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Competition across space: from metacommunities to social body-snatching trematodes

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Competition across space: from metacommunities to social body-snatching trematodes

by

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Dedication

To my parents

for leading me into ponds,

and to Andrius

for willingly following me into them.

Acknowledgements

Thanks to my committee and advisors, in all their iterations. Thanks to all my labs, official and unofficial, including the Leibold, Bolnick, Jha, Torchin, Hechinger, and Kuris/Lafferty labs. Thanks to Dr. Buck for her cages for Ch 3. Thanks to all the funding sources that helped me complete this work, including NSF EAGER Grant #1353919, an NSF GRFP, one of the last NSF DDIGs, and an NSF GRIP. Additionally, thanks to the IB department for a recruitment fellowship, and to UT Austin for a Harrington Dissertation Fellowship. Thanks to my fellow graduate students and friends, especially JELSAC and Maxine De Frreec, for keeping me sane during these past six years. And most importantly, thanks to my family for all of the emotional support!

Abstract

Competition across space: from metacommunities to social body-snatching trematodes

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The University of Texas at Austin, 2019

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This dissertation is composed of two sections. The first section focuses on experimentally testing the keystone community concept using protist microcosms. I found that habitat loss can cause structural changes in how communities are assembled, even when diversity measures appear unchanged. This work has important implications for reserves management and conservation efforts.

The second section is composed of three chapters on social body-snatching trematodes residing in the California horn snail. First, I investigated the tradeoff between reproduction and defense to determine if social trematode colonies increase their soldier investment in areas of high intraguild predation (IGP). I found that colonies appear to respond to IGP as predicted and do so at the site-level. Second, I conducted a reciprocal transplant experiment to determine if differences in soldier investment are due to phenotypic plasticity. Unfortunately, our results were inconclusive but provided us with valuable information on natural variation in these colonies within an estuary. Finally, I investigated how individual soldier attributes and colony composition might explain a linear competitive dominance hierarchy between six species of social body-snatching

trematodes. I found that the dominance hierarchy was not explained by attributes of the colony that we measured. All totaled, there are over 20,000 trematode species, in league with the diversity of social insect groups, like ants. The trematode system is rich with opportunity to study the evolution and ecology of sociality outside of insects.

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INTRODUCTION

Communities have historically been studied at the local scale from a 'niche-based' perspective, where processes driving community assembly are the abiotic environment and interspecific interactions (Hutchinson 1957, 1959, MacArthur 1969, Tilman 1982, Chase and Leibold 2003). From this perspective, communities are predicted to be explained primarily by local environmental factors; a prediction known as 'species sorting' (Chase and Leibold 2003). At larger scales, regional processes cause stochastic extinctions of local communities and connect these local communities through processes of dispersal, structuring communities less by local environmental conditions and more by spatial connectivity (Hanski and Gyllenberg 1997, Hubbell 2001, Leibold and Loeuille 2015, Resetarits and Silberbush 2015). Although most studies focus on a single scale, the interaction of regional processes (dispersal, demography, extinction) and local 'niche-based' properties for explaining community assembly is becoming increasingly apparent (e.g. Fagan 2002, Leibold et al. 2004, Cottenie 2005, Gravel et al. 2006, Leibold and McPeek 2006, Adler et al. 2007b). Approaches to studying this interaction include metacommunity ecology (Leibold et al. 2004, Holyoak et al. 2005, Leibold 2011, Logue et al. 2011), and landscape ecology (Troll 1939). Most work in metacommunity ecology focuses on this interaction between the local and regional scale in general terms and often ignores the spatial details of the metacommunity. In contrast, landscape ecology is focused on spatial context but often ignores more general community context such as patterns of biodiversity and the mechanisms of species interactions.

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[1] Resetarits, E. J., Cathey, S. E. and Leibold, M. A. (2018), Testing the keystone community concept: effects of landscape, patch removal, and environment on metacommunity structure. Ecology, 99: 57-67. doi:10.1002/ecy.2041. MAL conceived the project. EJR and SEC ran the experiment, EJR analyzed the results, and EJR wrote the manuscript.

How local patches or communities contribute to regional patterns is an important, and primarily unaddressed, question regarding the interaction of local and regional processes (Urban and Keitt 2001). At the community level, this question requires the integration of the spatially-explicit aspects of landscape ecology with metacommunity ecology. Mouquet et al. (2012) proposed the keystone community concept (KCC)—an extension of Robert Paine's (1966, 1969) keystone predator concept—which posits that the removal of some local patches may have disproportionate effects on the metacommunity. Mouquet et al.'s (2012) metacommunity-based models illustrate that particular local features of patches such as their size, productivity, or environmental distinctiveness can affect important features of the metacommunity such as its diversity or productivity. Economo (2011) and Gascuel et al. (2016) used neutral theory and landscape modeling to show that the particular spatial location of patches in a dispersal network could also affect overall diversity in complex ways even if there were no environmental differences among patches (see also Carrara et al. 2012). Thus both environmental features and position in a landscape could alter the contribution of individual patches to regional features of the metacommunity (Altermatt and Holyoak 2012, Carrara et al. 2014). Although the removal of a large or high complementary patch may have a strong impact on a metacommunity, this could be primarily due to direct effects, such as the loss of species in that single patch. However, in this study we are primarily interested in finding evidence of patches whose removal have significant indirect effects on the metacommunity resulting from downstream changes in neighboring patches.

The interaction of local and regional processes in shaping metacommunities is important not just for furthering ecological theory but also for environmental management. Habitat loss and fragmentation alter patterns of biodiversity and ecosystem attributes by changing the size and connectivity of the metacommunity, while habitat modification does so through changing the environmental conditions of patches. Such anthropogenic changes to both spatial and environmental context could interact to alter biodiversity and ecosystem services. The management of reserves and reserve networks

seeks to counter these anthropogenic effects. Current approaches to evaluate and conduct triage, and reserve selection are primarily focused on combining landscape ecology with metapopulation theory (e.g. Moilanen et al. 2005, Leroux et al. 2016, Albert et al. 2017). They often look to preserve low connectivity, rare patches that have higher endemism or high complementarity (unique species not found in the other patches; Economo 2011) or to try to maximize connectivity to promote long-term persistence (Margules and Pressey 2000). However, these approaches may be ineffective if the species involved have strong interactions that also depend on environmental and spatial context, making it imperative that a metacommunity perspective be used when possible. Identifying certain patch attributes that predict 'keystoneness' would be a critical tool for conservationists.

Protist microcosms are an ideal system for addressing conservation-related questions because they are well-studied, easily manipulated to mimic a variety of landscape types, and provide a needed bridge between ecological theory and on-the-ground conservation efforts. In this study, we used protist microcosms to experimentally test how the attributes of individual patches, including both location within the spatial network (high versus low connectivity), and environmental distinctiveness (rare versus common), altered properties of the metacommunity. We did this by comparing properties in a 'N patch' control metacommunity and in different 'N-1 patch' experimental metacommunities where we removed one patch only. Removal was done for all four combinations of high-vs-low connectivity and rare-vs-common environmental distinctiveness. We chose connectivity and environmental distinctiveness because they are local attributes dependent on regional context and because previous models suggest that these attributes should have indirect effects on the metacommunity (Mouquet et al. 2012, Fournier et al. 2016, Gascuel et al. 2016). Our metacommunity design combined the common elements of species sorting (habitat heterogeneity, niche partitioning between habitats, community assembly) with aspects of patch dynamics (background extinctions due to stochastic events and dispersal) and neutral theory (demographic stochasticity in small local populations). We focused on two main metacommunity properties. First, we examined the effect of patch removal on diversity and ecosystem

function metrics (average community richness, evenness, and biomass), and second, we used variation partitioning techniques (Borcard and Legendre 2002, Cottenie 2005, Peres-Neto et al. 2006, Logue et al. 2011) to assess the relative role of environmental conditions and spatial connectivity on community composition (referred to here as structuring processes). Additionally, we examined our results for evidence of 'keystone communities' by looking for patches that had a disproportionate effect on the metacommunity.

We hypothesized that highly connected, rare patches should have the largest effect on both diversity/ecosystem function measures and on structuring processes at the metacommunity level. The removal of highly connected patches should maximally disrupt dispersal/recolonization from neighboring patches and rare patches should harbor unique species found in a smaller proportion of communities and thus be more susceptible to regional extinction and stochasticity in recolonization (Wilson and MacArthur 1967, Levins 1969, Hanski 1999).

Our study found no evidence for the presence of 'keystone communities' and suggests that diversity measures (richness, evenness, biomass) of the metacommunity are robust to removal of single patches. However, we found that the removal of any single patch from a metacommunity caused community assembly to be driven less by environmental processes and more by spatial processes. Surprisingly, we found that landscape structure (i.e. the distribution of habitat types and stochastic extinctions across the metacommunity) had the largest effect on both diversity measures and structural processes. This study demonstrates the importance of assessing underlying regional processes in addition to local diversity measures in order to assess the impact of habitat loss at the regional scale.

MATERIALS AND METHODS

Experimental design

Experimental microcosms were optimal for testing metacommunity theory because they allowed us to directly compare the effects of removing different patches on identical

metacommunities. Our communities consisted of 6 protist species: *Colpidium striatium*, *Blepharisma sp., Euplotes sp., Philodina sp.,* and *Vorticella sp.,* which we ordered from Carolina Biological Supply (Burlington, NC), and *Paramecium sp.,* which was obtained from Dr. Jin Liang at Georgia Tech (Figure 1.1, Table 1.4). These species were used because they differ in characteristics that could affect their competitive ability, such as swimming speed, mean cell volume, and ontogenetic life stages, and because they vary in their preferred food source (bacteria versus algae), thus allowing us to have two different patch types that contained distinct communities.

Metacommunities were composed of a patchwork of two different habitat types, algal-based (autochthonous) and bacterial-based (allochthonous) as described by Fukumori *et al.* (2015). Each patch was a Qorpak 60ml jar containing 30 ml of COMBO culture medium (Kilham *et al.* 1998). The two habitat types differed in their source of organic matter (algal production or bacterial decomposition of organic matter) for the protists to subsist on. Algal patches were inoculated with 25,000 cells (1 mL) of *Chlamydomonas*. Algal patches were made of clear polystyrene plastic to allow for optimal light penetration. Bacteria patches had a degraded wheat seed added for rapid bacterial colonization from the environment. Bacterial patches were made of a white polypropylene plastic and were cultured under dark conditions to minimize light penetration and prevent algal growth. Both types of patches were cultured in the same culture chamber in order to hold all environmental parameters, except light conditions, constant.

Figure 1.1 – Microscope images of 6 protist species.

Top, left to right: *Blepharisma sp.*, *Colpidium striatum*, *Euplotes sp*. Bottom, left to right: *Paramecium sp.*, *Philodina sp.*, and *Vorticella sp.*

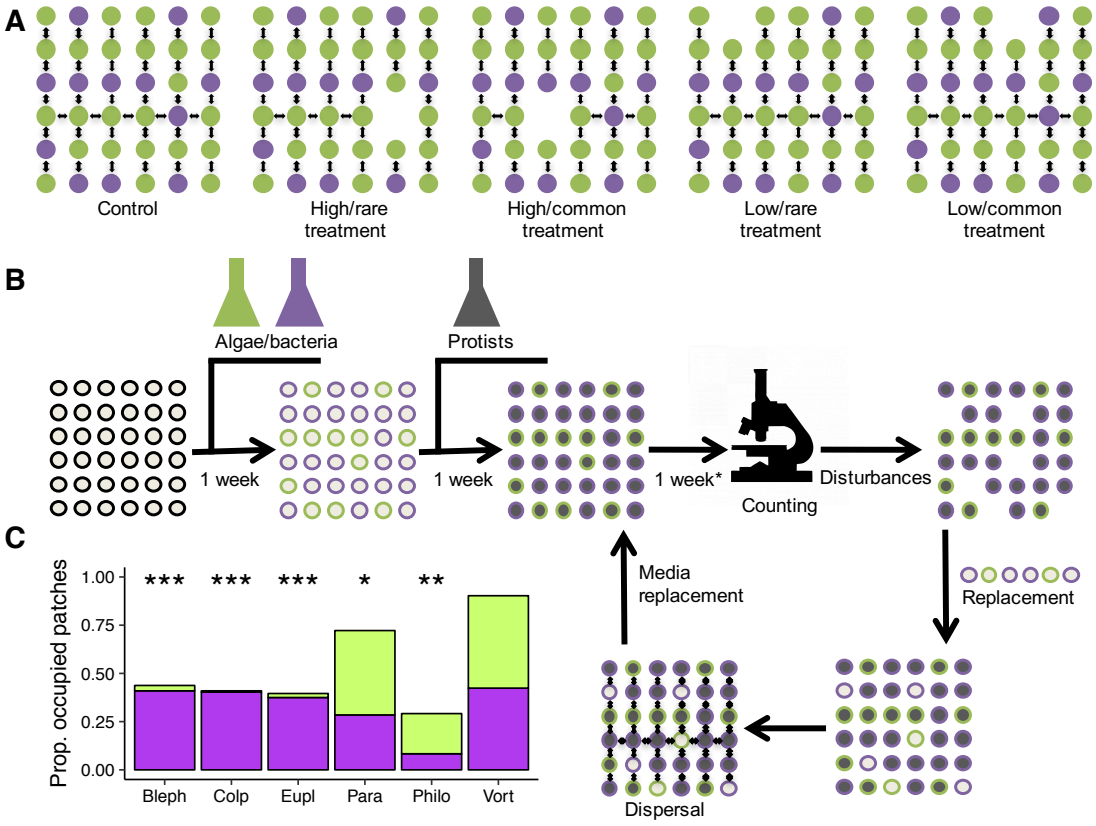

Figure 1.2 – Experimental design for testing keystone community concept

A) An example of an algal-dominated landscape, consisting of 5 replicate metacommunities (a control metacommunity and four treatment metacommunities) with identical extinctions and distributions of algal and bacterial patches. Arrows represent patches that are connected weekly by manual dispersal. Green circles represent algal patches and purple circles represent bacterial patches. The distribution of algal and bacterial patches varies between landscapes but are identical within a landscape. Our experiment contained 10 landscapes, half which were algal-dominated and half that were bacteria-dominated for a total of 1,760 patches and 50 metacommunities. B) Experimental design: a single metacommunity consisting of 36 uncolonized patches (black open circles) are inoculated with algae or bacteria and allowed to grow for one week. Colored circles represent algal-based patches (green open circles) or bacteria-based patches (purple open circles). Six species of protist are then added to each patch (filled circles) and allowed to grow for one week. After one week, counts are taken (for control metacommunities only). Six patches from each metacommunity are then removed as disturbances and replaced with patches that have either algae or bacteria, but no protists (green and purple open circles). Dispersal of protists between connected patches (arrows connect patches) then occurs, causing new, empty patches to be colonized (closed circles). 10% of the media in each patch was then replaced to allow communities to persist. Protists were then allowed to grow for a week before the process was repeated for 9-10 weeks. *Starts after first disturbance-replacement-disturbance cycle. **C)** The proportion of patches occupied by each of 6 protists (*Blepharisma* (Bleph)*, Colpidium* (Colp)*, Euplotes* (Eupl)*, Paramecium* (Para)*, Philodina* (Philo)*, Vorticella* (Vort)) across two habitat types based on week 1 data (pre-dispersal) for landscapes 7-10, representing species sorting for each species. 72 bacteria-based patches (dark purple) and 72 algal-based patches (light green) were inoculated with all 6 protists and allowed to grow for one week. Significant species sorting (differential survival between the two habitats) is indicated as follows: one asterisk, $p < 0.05$; two asterisks, $p < 0.01$; three asterisks, $p < 0.001$.

All replicate metacommunities had an identical 6x6 virtual dendritic structure and were each composed of 36 patches (Control) or 35 patches (Treatments; see Figure 1.2A). Metacommunities could either be algal-dominated (2/3^{rds} of the patches were algal-based) or bacteria-dominated (2/3^{rds} bacterial-based). In order to test the effect of patch removal on the metacommunity, each treatment consisted of the removal of a different "type" of patch. The patches we removed varied in both connectivity (high, low) and environmental distinctiveness (rare, common). Common patches had a habitat type that made up 2/3rds of the metacommunity, and rare patches had habitat types that made up 1/3rd of the metacommunity. The removal of high connectivity patches broke up the metacommunity into multiple unconnected sub-metacommunities, whereas the low connectivity patches did not. We therefore had four treatments, where a different type of patch was removed: high/common, high/rare, low/common, low/rare. These patches stayed empty for the entirety of the experiment, and we held their place with a container of ethanol (Figure 1.2A).

The distribution of algal and bacterial patches within the 6x6 metacommunity was randomly assigned but was identical within a landscape. A landscape was applied as a block treatment and consisted of 5 replicate metacommunities (a control metacommunity and four treatment metacommunities) with identical habitat distributions and extinctions (see below; Figure 1.2A). Our experiment contained 10 landscapes, half which were algal-dominated and half that were bacteria-dominated. We altered which habitat was dominant and the distribution of algal and bacteria-based habitat patches in order to maintain generality and to prevent our findings from being specific to one scenario. Our experiment used a total of 1,760 patches and 50 metacommunities.

Experimental protocol (Weekly)

Bacteria or algae were grown in patches for one week prior to the start of the experiment to ensure sufficient resources were available for protists (Figure 1.2B). We then inoculated each patch in the metacommunities with approximately 20 individuals of each protist species and left them to grow undisturbed for one week. Each week, we

replaced 3 ml (10%) from every patch with fresh COMBO media. We used the removed media to count protist densities (cells/mL) under a 10x light microscope for each patch in the control treatment. We used these weekly densities to monitor the progress of all the metacommunities within each landscape.

Disturbances, dispersal and media replacement occurred weekly in all metacommunities. Each week, to introduce stochastic disturbances into the system, we replaced six patches (four common, two rare) from each metacommunity with fresh patches that contained only COMBO medium and a source of organic matter (algae or bacteria) that had been growing for a week. These six patches were determined randomly, varied weekly, and were identical within a landscape. Dispersal was conducted after random patch disturbances to allow neighboring individuals to colonize the unoccupied patches. Dispersal was symmetrical and reciprocal between adjacent patches and was done manually by transferring 100 μL aliquots between virtually connected microcosms. One hundred microliters was chosen based on a pilot experiment because it produced moderate community richness, variation across the metacommunity (all species were not in all habitats), and allowed for spatial connectivity to be important without causing a large number of uncolonized (empty) patches (Figure 1.3).

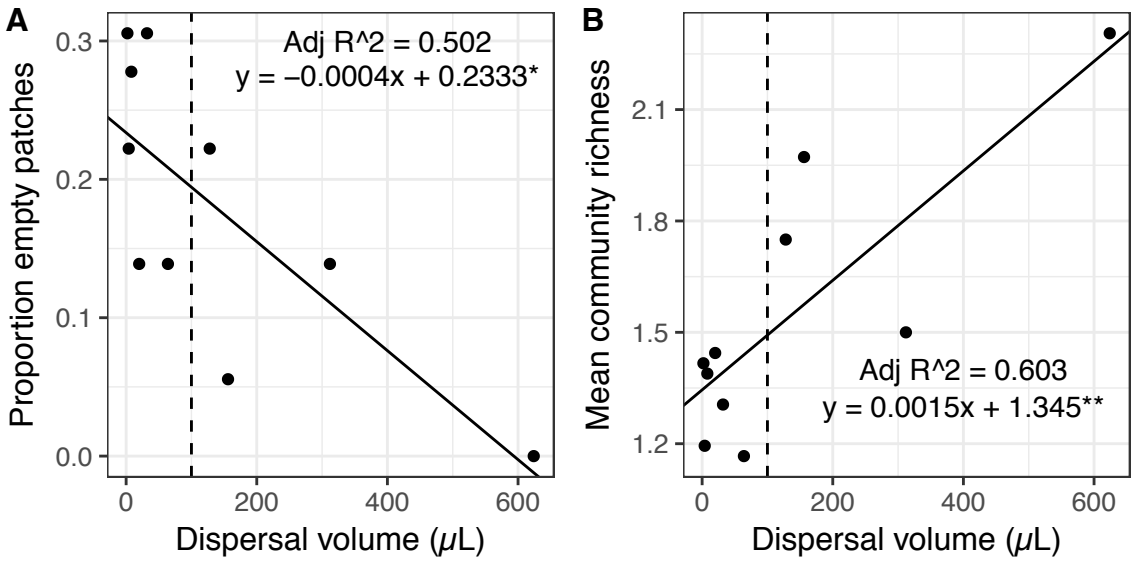

Figure 1.3 – The effect of dispersal volume on metacommunity

Effect of dispersal volume (μL) on **A**) the proportion of empty (i.e. uncolonized) patches and **B**) mean community richness and the dispersal volume chosen for pilot experiment (100μL; dashed line). There is a significant negative correlation between dispersal volume and the proportion of empty patches and a significant positive correlation between dispersal volume and mean community richness. Significant correlations are indicated as follows: one asterisk, $p < 0.05$; two asterisks, $p < 0.01$.

Final Counts

We performed experiments from January 2015-August 2015. We ran landscapes 1-6 starting in January (experiment 1) and ran landscapes 7-10 (experiment 2) starting in May. Each experiment ran for 9-10 weeks, representing over 60 generations for most of the protist species. Algal-dominated landscapes were counted at 9 weeks and bacteria-dominated habitats were counted at 10 weeks due to time constraints and sampling intensity. Final counts were recorded as density (cells/mL) per species for each patch.

Diversity measures

In order to assess how removing certain patches from a metacommunity might affect diversity and ecosystem functioning, we calculated three broad community level measures: average community richness, evenness, and biomass. We used average community level measures in order to control for the direct effects of removing a patch on the metacommunity (36 to 35 patches) and to focus on the indirect effects. Richness was calculated as the average number of species found in each patch. Richness was not rarified because we scanned a larger volume of media for low density patches (<10 individuals) in order to detect rare taxa. Furthermore, because maximum richness of a patch is known (6 species), rarefaction would potentially give us unrealistically high richness (>6 species). Community evenness was assessed by calculating Shannon's diversity (H') using the R package vegan and dividing it by the natural log of the richness.

Biomass was calculated by multiplying the density (cells/mL) of each species by an average volume/cell for that species. We calculated average volume for each species by photographing 10 specimens of each species (of varying size) from our original cultures, measuring length and width using ImageJ, and then applying the equation for the volume of an ellipsoid. *Paramecium* and *Vorticella* cultures used for biomass calculations were primarily grown on *Chlamydamonas* while *Blepharisma*, *Euplotes*, *Philodina*, and *Colpidium* were grown on bacteria. Size did vary between environmental conditions for *Blepharisma*, *Euplotes*, and *Colpidium*, but this was not taken into account since these species were uncommon in algal patches and only in low densities. Volume estimations were then averaged across the ten replicates to get an average volume/cells of each species. Biomass is expressed as the volume of protists per mL of media. All analyses, except variation partitioning, were done in R version 3.3.1 (Bug in Your Hair).

Variation partitioning

In order to determine the relative importance of spatial and environmental processes for structuring communities, we used variation partitioning techniques

(Borcard and Legendre 2002, Cottenie 2005, Peres-Neto et al. 2006). The environmental parameters used were habitat type (algal or bacterial) and age since most recent disturbance. In order to detect complex spatial patterning, we used Moran's Eigenvector Maps, which uses a weighted connectivity matrix to extract eigenvectors that maximize Moran's index of autocorrelation (Dray *et al.* 2006). We used a binary weights matrix and only included significant eigenvectors with positive autocorrelation.

Variation partitioning was performed for all 50 metacommunities at the end of the experiment to assess how well space (significant positive eigenvectors) and the environment (habitat type, age) explained community composition across the metacommunity (Borcard and Legendre 2002, Cottenie 2005). Raw community data was Hellinger transformed using the vegan package for all subsequent analyses (Legendre and Gallagher 2001). Redundancy Analyses (RDA) were done to test the significance of each component. Non-significant components for a metacommunity were given a value of zero in the data table used for subsequent analyses. However, including these non-significant components in the analyses did not qualitatively change the results, because non-significant components had very low values. R version 3.0.2 (Frisbee Sailing) and packages spacemakeR and packfor were used for variation partitioning analyses.

Statistical analyses

In order to assess habitat preferences for our six species, we calculated the number of algal and bacterial patches that possessed a given species at week 1 (after 1 week of protist growth). This measurement was only done for landscapes 7-10 due to misidentification of *Vorticella/Philodina* species for landscapes 1-6 during the first weeks of counting. We used a Pearson's chi-squared test to assess if species were found more often in one habitat than would be expected (50:50), given all species were inoculated into all patches.

We used a nested ANOVA to determine the effect of removing any patch (treatments versus control) on each of our metacommunity properties (e.g. richness or environmental component) where landscape is nested within dominant habitat (Model:

metacommunity property ~ treatment + experiment + landscape(dominant habitat) + treatment*dominant habitat). For metacommunity properties that were significantly affected by removing any patch, we looked to see if there was variation between treatments. In order to compare metacommunity properties between treatments, we standardized measurements for each treatment by the corresponding control in that landscape. This gave us an effect size for a given treatment (i.e. of a given patch) and allowed us to test for 'keystone communities'. One-way ANOVAs were done using this standardized dataset to see if there were differences between treatments (Model: effect size of metacommunity property ~ treatment). A significant difference in effect size between a single treatment and all other treatments signified a 'disproportionate effect' – i.e. a 'keystone community'.

Additionally, in order to investigate what aspects of our landscapes significantly altered metacommunity properties (see Results), we assessed spatial autocorrelation and Weighted Recovery Time post-hoc. We assessed spatial autocorrelation (i.e. how well the two habitat types were dispersed throughout the landscape) using Moran's I. We calculated Weighted Recovery Time since disturbance using the equation,

$$\frac{\sum_{i=1}^{n} a_i * c_i}{n} \quad (1)$$

where a_i is the age of a patch (i.e. time since last disturbance), c_i is the connectivity of the patch (0-4), and n is the number of patches in the metacommunity (35 or 36). In this equation, older or more connected patches contribute more to Weighted Recovery Time than newly disturbed or less connected patches. One-way ANOVAs were used to see if there were differences in Moran's I and Weighted Recovery Time between landscapes using all 50 metacommunities (Model: e.g. Moran's I ~ landscape). Pearson product-moment correlations were used to look for relationships between each metacommunity property (e.g. richness, environmental component) and Moran's I or Weighted Recovery Time.

RESULTS

Species environmental preferences

We found that protist taxa had different preferred habitats. *Blepharisma sp.* (χ^2 = 48.016, df = 1, p-value < 0.0001), *Colpidium striatum* (χ^2 = 55.068, df = 1, p-value < 0.0001), and *Euplotes sp.* (χ^2 = 45.632, df = 1, p-value < 0.0001) were primarily found in bacterial patches, whereas *Paramecium sp.* (χ^2 = 4.654, df = 1, p-value = 0.031) and *Philodina sp.* (χ^2 = 7.714, df = 1, p-value = 0.005) were primarily found in algal patches (Figure 1.2C). The only species that did not appear to have a habitat preference was *Vorticella sp.*, which was the most common species across both habitats (χ^2 = 0.492, df = 1, p-value = 0.483).

Table 1.1 – Summary of diversity, ecosystem function, and variation partitioning results

Data summary for diversity, ecosystem function measurements and variation partitioning for control and treatments landscapes. Evenness was calculated by dividing Shannon's diversity (H') by the natural log of the richness. Biomass was measured in mL protist per mL media.

		n	Mean	SE	Min	Max
Diversity and Ecosystem Function						
Biomass	Control	10	5.358E-05	5.580E-06	2.294E-05	7.710E-05
	Treatment	40	4.886E-05	3.076E-06	2.086E-05	9.648E-05
Evenness	Control	10	0.437	0.017	0.375	0.545
	Treatment	40	0.429	0.011	0.249	0.625
Richness	Control	10	2.772	0.143	2.306	3.556
	Treatment	40	2.723	0.060	1.857	3.400
Variation Partitioning (%)						
Environmental (E|S)	Control	10	26.668	2.584	12.316	38.281
	Treatment	40	20.303	1.604	0.000	42.761
Spatial (S|E)	Control	10	11.906	3.504	0.000	28.413
	Treatment	40	19.061	1.769	0.000	40.183
Joint (EwS)	Control	10	10.864	3.323	0.000	29.354
	Treatment	40	11.818	1.077	0.000	23.538

Landscape effects

There was considerable variation among our controls in terms of diversity measures and variation partitioning results (Table 1.1; Figure 1.4A). Some of the variation in our diversity measures was due to differences between our two experiments (landscapes 1-6 versus landscapes 7-10; Table 1.2). Another reason for this variation was that bacteria-dominated metacommunities exhibited significantly higher average community evenness ($F=6.532$, $p < 0.05$), average community biomass ($F = 9.546$, $p < 0.01$), and were more spatially structured than algal-dominated metacommunities ($F = 9.157$, $p < 0.01$; Table 1.2; Figure 1.4A). Our landscapes (i.e. block treatments), which each had a different distribution of algal and bacterial patches and had different weekly extinctions, significantly differed in average community richness ($F = 8.739$, $p < 0.0001$), average community evenness ($F = 2.990$, $p < 0.05$), the purely environmental (E|S) component ($F = 7.840$, $p < 0.0001$), and the joint environmental-spatial (EwS) component ($F = 6.463$, $p < 0.0001$). However, landscapes did not significantly vary in the purely spatial (S|E) component ($F = 1.864$, $p = 0.103$) or in average community biomass ($F= 1.421$, $p = 0.225$). Although our study was not designed to tease apart how different aspects of spatial arrangement may influence diversity measures and underlying processes, we found that Moran's I (spatial autocorrelation) and Weighted Recovery Time were significantly different between landscapes ($F = 33.080$, $p < 0.0001$; $F = 7.601$, $p < 0.0001$; Table 1.3). Additionally, Moran's I was negatively correlated with average community biomass ($t = -4.687$, $df = 48$, $p < 0.0001$) and richness ($t = -2.584$, $df = 48$, $p < 0.05$; Figure 3B), and Weighted Recovery Time was positively correlated with average community biomass ($t = 4.287$, $df = 48$, $p < 0.0001$), richness ($t = 2.372$, $df = 48$, $p < 0.05$), and evenness ($F = 2.594$, $p < 0.05$; Figure 1.4C). No significant correlations were found between these landscape metrics and variation partitioning results (Table 1.3).

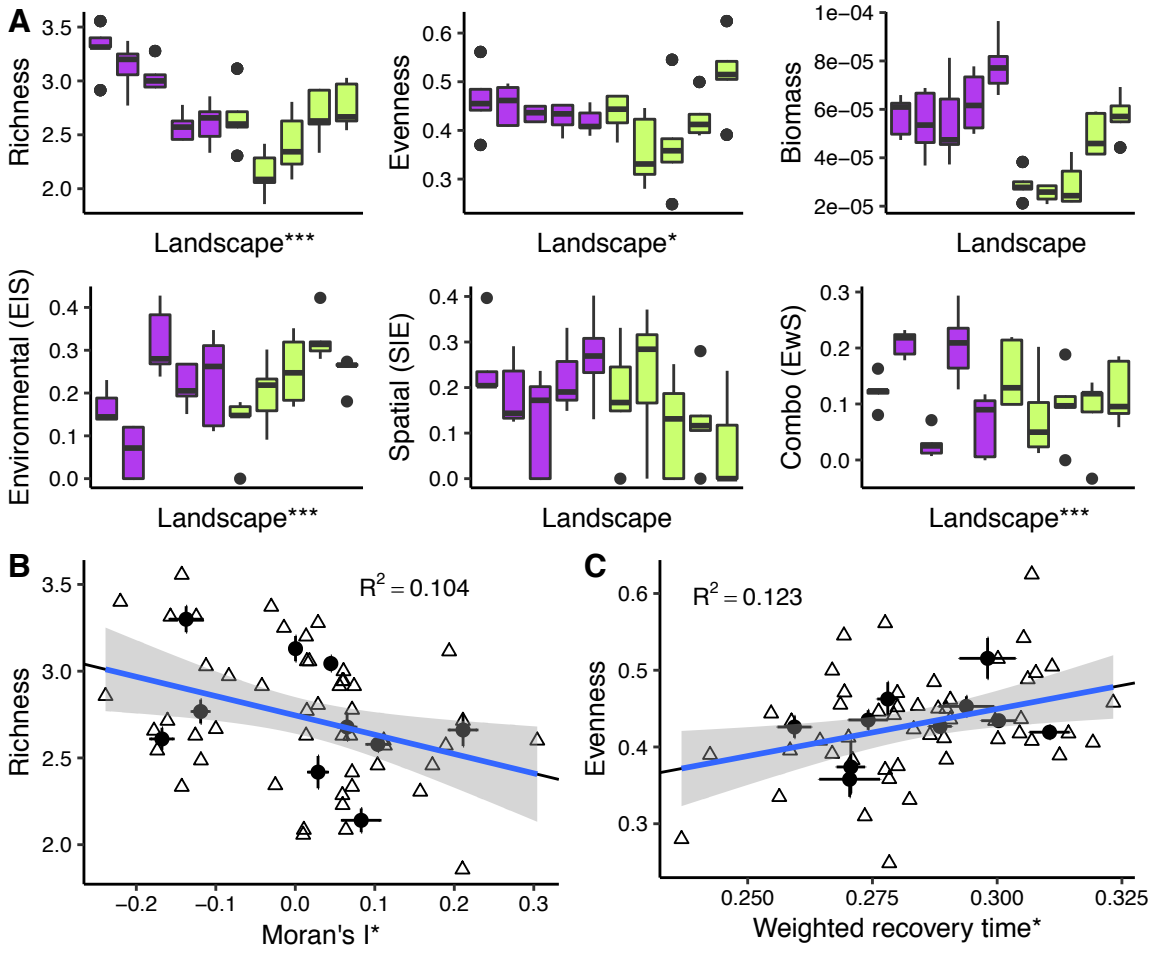

Figure 1.4 – Significant differences between landscapes

A) Boxplots showing the variation of diversity measures (Top: richness, evenness, and biomass) and variation partitioning results (Bottom: environmental component, spatial component, and joint) across our ten landscapes. Dark purple boxplots represent bacteria-dominated landscapes and light green boxplots represent algal-dominated landscapes. **B)** The effect of spatial autocorrelation (Moran's I) on average community richness and **C)** Weighted Recovery Time on average community evenness. Filled circles with error bars represent means and standard errors for each landscape for both parameters, showing that variation within landscapes is small. Open triangles represent individual metacommunities. Correlation coefficients and significance are based on individual metacommunity measurements. Shaded areas represents the 95% confidence interval. Moran's I varies between -1 (overdispersion) and 1 (positive autocorrelation). Significance effects are described as follows: period, $p < 0.1$; one asterisk, $p < 0.05$; two asterisks, $p < 0.01$; three asterisks, $p < 0.001$.

Table 1.2 – Model results for diversity, ecosystem function and variation partitioning

ANOVA results for diversity, ecosystem function and variation partitioning measurements (Model: factor ~ treatment + experiment + landscape(dominant habitat) + treatment*dominant habitat).

Factor	Sum Sq	DF	F value	P
Richness				
Treatment	0.019	1	0.373	0.545
Experiment	1.191	1	22.802	**<0.0001**
Dominant Habitat	0.063	1	1.197	0.281
Landscape	3.195	7	8.739	**<0.0001**
Treatment*Dominant Habitat	0.056	1	1.076	0.306
Evenness				
Treatment	4.59E-04	1	0.130	0.721
Experiment	4.68E-03	1	1.321	0.258
Dominant Habitat	2.31E-02	1	6.532	**0.015**
Landscape	7.42E-02	7	2.990	**0.013**
Treatment*Dominant Habitat	9.47E-03	1	2.674	0.110
Biomass				
Treatment	1.790E-10	1	1.536	0.223
Experiment	1.117E-09	1	9.609	**0.004**
Dominant Habitat	1.110E-09	1	9.546	**0.004**
Landscape	1.156E-09	7	1.421	0.225
Treatment*Dominant Habitat	2.200E-12	1	0.019	0.892
Environmental (E|S)				
Treatment	0.032	1	7.741	**0.008**
Experiment	0.010	1	2.375	**0.132**
Dominant Habitat	0.098	1	0.219	0.642
Landscape	0.139	7	7.840	**<0.0001**
Treatment*Dominant Habitat	0.001	1	1.185	0.283
Spatial (S|E)				
Treatment	0.041	1	3.841	0.057
Experiment	0.001	1	0.096	0.758
Dominant Habitat	0.098	1	9.157	**0.004**
Landscape	0.139	7	1.864	0.103
Treatment*Dominant Habitat	0.001	1	0.070	0.793
Combo (EwS)				
Treatment	7.29E-04	1	0.220	0.642
Experiment	8.57E-03	1	2.589	0.116
Dominant Habitat	7.88E-03	1	2.379	0.131
Landscape	0.150	7	6.463	**<0.0001**
Treatment*Dominant Habitat	1.93E-04	1	0.058	0.811

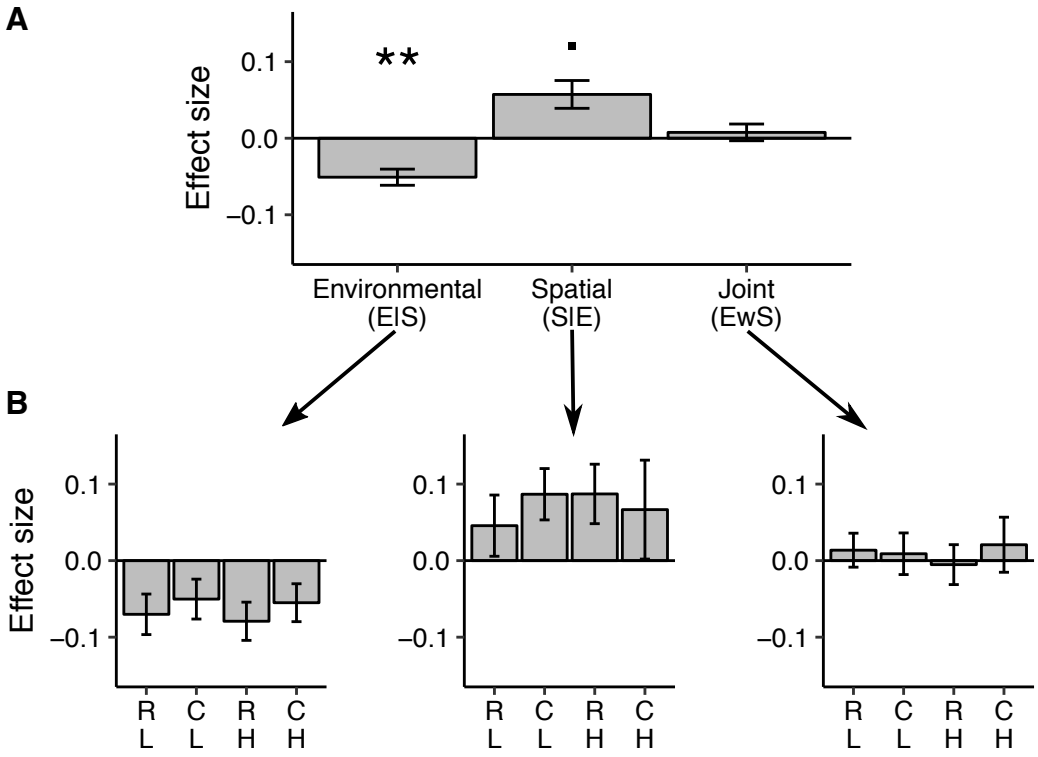

Figure 1.5 – Effect of treatments on structural processes

A) Bars represent the mean effect size (Treatment – Control) on the environmental, spatial, and joint environmental-spatial components when removing any patch from the metacommunity. Removing a patch increased the spatial component and decreased the environmental component. B) Bars represent and the effect sizes of individual treatments for the environmental, spatial, and joint environmental-spatial component. The top row of the x axis represents the commonality of each treatment: rare (R) or common (C). The bottom row of the x axis represents the connectivity of each treatment: low (L) and high (H). Error bars represent standard error. Significance levels for the effect size of removing a community are as follows: period, $p < 0.1$; one asterisk, $p < 0.05$; two asterisks, $p < 0.01$; three asterisks, $p < 0.001$.

What is the effect of removing any patch?

There was no significant effect of removing a patch on average community richness ($F = 0.373$, $p = 0.545$), evenness ($F = 0.130$, $p = 0.721$), or biomass ($F = 1.536$, $p = 0.223$) (Table 1.2). However, we did find that removing any patch significantly decreased the purely environmental (E|S) component ($F = 7.741$, $p = 0.008$), which is how much

local environmental factors determined community composition, and nearly significantly increased the purely spatial (S|E) component, which is how much stochastic extinctions and dispersal between connected patches determined community composition ($F = 3.841$, $p = 0.057$; Table 1.2; Figure 1.5A). On average, removing a patch decreased the variation explained by the purely environmental component from 26% to 20%, and increased the variation explained by the spatial component from 12% to 19% (Table 1.1). The joint environmental-spatial (EwS) component was, however, not impacted by removing any patch ($F = 0.220$, $P = 0.642$). There was no significant interaction between treatment and dominant habitat for any of our measurements, meaning that we did not find differing effects of treatment for algal and bacterial dominated metacommunities.

Table 1.3 – Correlations between landscape metrics and metacommunity properties

Table of correlations between two landscape metrics (Moran's I and Weighted Recovery Time) and six metacommunity properties (biomass, evenness, richness, environmental component, spatial component, and joint environmental-spatial component).

	Metric	t	df	p-value	r	R^2
Diversity and Ecosystem Function						
Total Biomass	Moran's I	-4.687	48	**< 0.0001**	0.560	0.3136
	Weighted Recovery Time	4.287	48	**< 0.0001**	0.526	0.2767
Evenness	Moran's I	-1.909	48	0.062	0.266	0.0705
	Weighted Recovery Time	2.594	48	**< 0.05**	0.351	0.1229
Richness	Moran's I	-2.584	48	**< 0.05**	0.349	0.1218
	Weighted Recovery Time	2.372	48	**< 0.05**	0.324	0.1048
Variation Partitioning (%)						
Environmental (E|S)	Moran's I	-0.266	48	0.791	0.038	0.0015
	Weighted Recovery Time	0.101	48	0.920	0.015	0.0002
Spatial (S|E)	Moran's I	-0.392	48	0.697	0.057	0.0032
	Weighted Recovery Time	-0.425	48	0.673	0.061	0.0038
Joint (EwS)	Moran's I	0.781	48	0.439	0.112	0.0125
	Weighted Recovery Time	-0.015	48	0.921	0.014	0.0002

Are there keystone communities?

There were no significant differences in effect size (control – treatment) between treatments for either the environmental (E|S) component (F-value = 0.275, df =3, p-value = 0.843), the spatial (S|E) component (F-value = 0.185, df = 3, p-value = 0.906), or the joint environmental-spatial (EwS) component (F = 0.244, p = 0.865; Figure 1.5B). Thus, we found no patch type, based on connectivity and commonality, that exhibited keystoneness for any of our metacommunity attributes.

DISCUSSION

Most work on metacommunities has focused on how regional processes affect local patch properties (e.g. diversity or productivity; Mouquet and Loreau 2003, Economo and Keitt 2008). However, understanding how individual patches affect metacommunity-wide processes is key for predicting changes in biodiversity measures over long time scales and has been relatively understudied (Mouquet et al. 2012, Gascuel et al. 2016). In this study, we focused on how removing certain patches affected metacommunity-level diversity patterns and structural processes. We found that the metacommunity, in general, was highly resistant to the removal of a community, in terms of biodiversity and ecosystem function measures. On the other hand, we found that removing a patch did have a strong effect on underlying structural processes as identified by variation partitioning. Removing any patch decreased the importance of environmental filtering and increased the importance of spatial structure and dispersal for driving community assembly across the metacommunity.

Unexpectedly, we found that the strongest driver of our communities was our landscape (i.e. block) treatments. Landscapes differed in their distribution of algal and bacterial-based patches and the location of weekly stochastic patch disturbances. These effects were substantially larger than the removal of any single patch (and the concomitant dispersal linkages) and were not due to differences in metacommunity size, connectivity, or average disturbance regime (all controlled or randomized in our experimental design) and presumably reflect particular details of the landscape

arrangement of patches. Although our cursory investigation suggests that spatial autocorrelation of habitat types (Moran's I) and Weighted Recovery Time since disturbance may be important for community diversity and ecosystem functioning, the relative importance of each cannot be determined from this study. Additionally, much of the variation between landscapes, especially in environmental and spatial structuring, was not explained by either parameter. More nuanced metrics than those used here will likely be required to tease apart these landscape effects further.

One of the goals of this study was to identify keystone communities—patches that have a disproportionate effect on the metacommunity when removed (Mouquet *et al.* 2012). Although removing a patch did have a significant effect on the metacommunity (increase in spatial component, decrease in environmental component), there was no difference in the effect size based on what type of patch was removed (high/rare, high/common, low/rare, low/common). This is surprising, since removing a high connectivity patch broke up our metacommunities into smaller, disconnected metacommunities, and a large body of theory steming from island biogeography and metapopulation ecology suggests that this should have a negative impact on species diversity and richness (Wilson and MacArthur 1967, Levins 1969, Urban and Keitt 2001). Not finding evidence for keystone communities in this case certainly does not mean that they do not exist in other contexts. One reason there may not have been keystone communities in our system is that our metacommunities did not have enough habitat variability to produce patches with either unique attributes (e.g. super high nutrients ; Mouquet *et al.* 2012) or high enough diversity to produce patches with unique species (Economo 2011). Additionally, the low species diversity of our system, combined with the large metacommunity size, may have dampened effects that would have been more pronounced given a smaller metacommunity and/or more diverse community. However, our results suggest that keystone communities, if found, may still have rather subtle effects.

Although in our experiment we omitted patches from a given metacommunity, what we were really interested in was the effects of patch removal. We do not believe

that our results would have qualitatively changed if we had allowed full metacommunities to establish and then removed a patch rather than simply omitting a patch from the beginning. The reason being that omission and removal experiments should only differ if the starting conditions (i.e. species distributions across the metacommunity) differ. If, for example, we had inoculated only a few patches to begin with, or had some spatial community structure from the beginning, one could imagine removing a patch from the metacommunity could have different downstream (so to speak) effects than the omission of a patch. However, since we inoculated every patch with the same 'cocktail' of species, we have not induced any spatial structure into our metacommunities that should cause omission of a patch to differ from the removal of a patch.

Our results support previous work showing that higher patch extinctions within a metacommunity cause a decrease in environmental structuring and an increase in spatial structuring (Fukumori et al. 2015, Leibold and Loeuille 2015). Additionally, the variation explained by both environment and space in our experiment mirror the results from low and medium dispersal treatments of Fukumori et al. (2015) which also had two distinct habitat types but had a grid-like spatial layout. We found that the loss of a single patch, which constituted less than a 3% (1/36) change in the size of the metacommunity, could significantly alter the fundamental processes structuring that metacommunity by decreasing the environmental component by roughly 20% and increasing the spatial component by roughly 50%. A decrease in the environmental component signifies a decrease in 'species sorting' (Leibold *et al.* 2004), which can alter ecosystem functioning (Leibold et al. 2017). Taken together with previous work these findings suggest that as anthropogenic forces disconnect or chip away at suitable wildlife habitat, the processes structuring these communities may be quickly changing in large, important ways, even if diversity and ecosystem properties appear unchanged.

Many conservation efforts focus on preserving habitat patches with high complementarity (i.e. that have unique species), and these patches are often those that are environmentally distinct and poorly connected to other patches (e.g. islands; Margules

and Pressey 2000). Using a neutral metacommunity (i.e. solely structured by space), Economo found that in the long term, both highly connected (redundant) and poorly connected (complementary) patches had the same long-term effects on diversity. In other words, Economo found that when metacommunities were driven solely by spatial structure (neutral), metacommunity diversity was directly related to metacommunity size, suggesting that conservation strategies to maintain species diversity may be ineffective if habitat loss is unavoidable. In non-neutral metacommunities (structured by both the environment and space), we found that the loss of any patch caused the metacommunity to become more structured by space. If species in metacommunities that are more structured by space are harder to conserve because of a strong causal relationship between metacommunity size and diversity, and a decrease in metacommunity size also causes an increase in the importance of spatial structure, this suggests that conservation efforts may become increasingly ineffective as habitat quantity decreases. Of course, more work across scales and different systems is needed in order to confirm this relationship between metacommunity size and spatial structuring before we can assess how applicable these results are to conservation.

As our questions and concerns become more global or large-scale, rigorous experiments are becoming less and less tractable. Especially for metacommunities, we have come to rely more and more on modeling, data mining, and observational studies rather than empirical testing. However, without rigorous experimentation, we lack the ability to identify intrinsic mechanisms and assess causality (Benton et al. 2007, Paine 2010). Fortunately, microcosm and mesocosm experiments using model organisms can allow us to directly address seemingly-intractable global questions (Carrara et al. 2012, Altermatt and Holyoak 2012, Carrara et al. 2014, Fukumori et al. 2015).

As this study demonstrates, there is an ever-increasing need to include the role of spatial context and scale for understanding community and metacommunity dynamics (Schiesari et al. 2002, Bengtsson 2009, Leibold and Chase 2017). Although many studies focus on the impacts of disturbances on diversity, and the effects of diversity on community and ecosystem processes, fewer have focused on how disturbances effect

underlying processes structuring communities. Our study clearly shows that even without changes in more traditional community measurements (diversity, etc.), there can still be strong structural changes occurring in the metacommunity. Future work will need to focus on understanding the role of these changes to underlying processes on long-term community diversity and stability.

Table 1.4 – Table of functional and morphological differences between protist species used in this experiment.

Body size mean and standard error for each species based on photographs of 10 specimens. Reproduction and other defining characteristics based on Jahn et al. (1978) and Lynn (1979).

Species	Mean Body Size (mL)	SE Body Size (mL)	Mean Body Length (μm)	Body shape	Motility	Reproduction	Habitat preference	Feeding preference	Other defining characteristics
Blepharisma sp.	5.373E-07	9.644E-08	298.634	Ellipsoid or pyriform	Free-swimming	Fission; conjugation	Bacterial patches	Bacteria, microalgae, and other ciliates	Pink cytoplasmic pigment, which can be lethal to predators; larger organisms can be cannibalistic
Colpidium striatum	5.489E-08	8.414E-09	88.402	Elongate-ovoid	Free-swimming	Fission	Bacterial patches	Bacteria	
Euplotes sp.	3.685E-07	4.271E-08	120.646	Ovoid, dorso-ventrally flattened	Free-swimming	Fission; conjugation	Bacterial patches	Bacteria and microalgae	substrate-oriented
Paramecium sp.	7.240E-08	6.847E-09	88.496	Slipper-shaped	Free-swimming	Fission; conjugation	Algal patches	Bacteria and microalgae	
Philodina sp.	3.055E-07	6.730E-08	186.768	Elongate	Free-swimming	Parthenogenesis	Algal patches	Bacteria and microalgae	Can contract corona into body
Vorticella sp.	2.358E-08	2.287E-09	43.440	Bell-shaped to cylindroid	Free-swimming; aggregations	Fission; conjugation	None	Bacteria and microalgae	Stalked bell-shaped life stage

Chapter 2: Body snatching social trematodes allocate defense according to intra-guild predation intensity in the field.

INTRODUCTION

Social organisms that live in colonies with a caste division of labor are found throughout the animal kingdom, including hymenopteran insects (Oster and Wilson 1978), snapping shrimp (Duffy 1996), naked mole rats (Jarvis 1981, O'Riain et al. 2000), anemones (Ayre and Grosberg 1996; Francis 1976), and trematodes (Hechinger et al. 2011). Despite their diversity, many experience key tradeoffs in allocating resources to different castes (Oster and Wilson 1978). Soldier production, for example, comes at the cost of colony reproduction, since investment in non-reproductive soldiers decreases colony reproductive output (Passera et al. 1996, Kamiya et al. 2013, Lagrue et al. 2018). Indeed, Oster and Wilson (1978) argue that colonies should be able to tailor their investments in reproduction and defense in response to environmental factors such as resource availability, predation, and competition to maximize total reproductive output (Oster and Wilson 1978). However, there is limited work investigating whether colonies can alter their caste allocation in response to changing environmental conditions, such as increased predation or competition (Passera et al. 1996, Aguilera-Olivares et al. 2017). This is partly due to the difficulty in quantifying both species' interaction strengths (e.g. the strength of competition or predation that a focal species/colony experiences) in nature and censusing whole colonies (Palmer 2004).

Here, we focus on intraguild predation (IGP) because it is a powerful and ubiquitous interaction which incorporates predation and competition and has been shown to alter species' resource use (Hechinger 2010). The diverse guild of body-snatching trematodes that infect the California horn snail are an excellent system to examine how IGP intensity influences caste investment for several reasons. First, the colonial stages (rediae) of several trematode species have been shown to have castes, with large reproductives and smaller, non-reproductive soldiers (Hechinger et al. 2011, Garcia-Vedrenne et al. 2016, 2017). This soldier caste is devoted to defense against other

invading trematodes, readily using their mouthparts to attack and ingest both interspecific and intraspecific (but in a different colony) trematode colonial stages (Hechinger et al. 2011, Garcia-Vedrenne et al. 2016, 2017), and move to the front of the snail where trematodes invade. Second, the strength of IGP is well-documented in this system (Kuris and Lafferty 1994, Kuris 1990, Sousa 1993, Lafferty et al. 1994), and is strong enough to influence community structure and drive adaptive allocation of resources between growth and reproduction (Hechinger 2010). Third, these trematode species follow a well-understood linear IGP hierarchy (Kuris 1990, Huspeni 2000). Finally, these trematodes are widely dispersed via shorebird final hosts, such that they have little genetic spatial structure (Miura et al. 2006, Keeney et al. 2009). This wide dispersal (i.e. high gene flow) and low population structure suggests that selection would need to be extremely strong for local adaptation to occur.

Previous studies have hinted at the ability of interaction strength to affect caste ratios. For instance, caste ratio (soldiers:reproductives) was significantly higher in one site with high infection prevalence (a metric of IGP intensity) relative to two sites with low prevalence in a similar snail-trematode system (Lloyd and Poulin 2014). Similar studies have been done in hymenoptera and have found that colonies show higher caste ratios in more tropical regions, where competition is higher (Wills et al. 2014). However, the small number of localities in all of these past studies preclude detection of a potential relationship between caste ratio and interaction strength. Additionally, although coinfections are rarer than would be expected due to high intraguild predation (Kuris 1990, Sousa 1993, Kuris and Lafferty 1994, Lafferty et al. 1994), it has been shown that trematode colonies increase soldier investment when their snail host is coinfected by another trematode species (Lloyd and Poulin 2013b, Mouritsen and Andersen 2017, MacLeod et al. 2018, Lagrue et al. 2018). However, attempts to show plasticity in caste ratio in response to external changes in IGP intensity through lab experiments and common garden experiments have failed to do so (Lagrue et al 2018; Lloyd and Poulin 2014)

Here, we present the first quantification of how soldier investment varies with IGP intensity. We utilize a previously-described latitudinal gradient of IGP intensity (Torchin et al. 2015) to test whether trematode colonies follow optimal caste allocation theory by investing in soldier defense in proportion to IGP intensity (Oster and Wilson 1978). The California horn snail and its trematode parasites have a large latitudinal range (Torchin et al. 2015; Hechinger et al unpublished), with IGP intensity highest around southern California and declining going both north and south (Torchin et al. 2015). This non-linear relationship allows us to disentangle the impact of IGP intensity from other variables that covary linearly with latitude, such as temperature. Here, we quantify IGP intensity using infection prevalence as a proxy (Torchin et al. 2015) at multiple spatial scales to investigate three predictions. Specifically, we predict that 1) colonies facing higher IGP intensity should invest more in soldier defense, 2) these colonies should deploy more of these soldiers to the front of the snail where invasions occur, and 3) the strength of these relationships should vary between trematode species such that species respond differently to IGP intensity based on their competitive rank. We also provide the first evaluation of soldier investment with geographic variation in interaction strength (IGP, parasitism, competition) in any eusocial taxa.

MATERIALS AND METHODS

Target species collection

We focused on six trematode species that infect California horn snails, *Cerithideopsis californica,* chosen because they possess soldiers and vary in their competitive rank (Table 2.1; Mordecai et al. 2016). Snails were collected from nine estuaries across California and three estuaries across Panama (Table 2.4). We note that the California horn snail includes three nominal species (*C. californica*, *C. mazatlantica* and *C. valida*) but is considered here as a single, polymorphic species based on identical mitochondrial haplotypes and the same 28S genotypes (Miura et al 2010).

At most locations, 100 snails were collected during low tide from three sites per estuary (300 snails per estuary). However, in some estuaries in Panama, California horn

snails are very patchy and only occurred in small areas, so all 300 snails were taken from one site (Table 2.5). A total of 5520 snails were dissected to estimate infection prevalence and infer IGP intensity across this geographic range (see next section).

Identification and colony processing

In the laboratory, snails were measured with Vernier calipers, processed following Torchin et al (2005), and trematode species were identified following Martin (1972) and Hechinger and Huspeni (unpublished manuscript). We assessed soldier allocation and deployment for snails that had mature, single-species infections of a trematode of interest (Table 2.1). These snails were extracted from their shell, divided into three sections (the mantle, the middle, and the gonadal region), and soldiers and reproductives were counted in each section (Hechinger et al. 2011; Figure 2.1). This allowed us to estimate soldier investment (total soldiers) and deployment (proportion of soldiers in the invasion front).

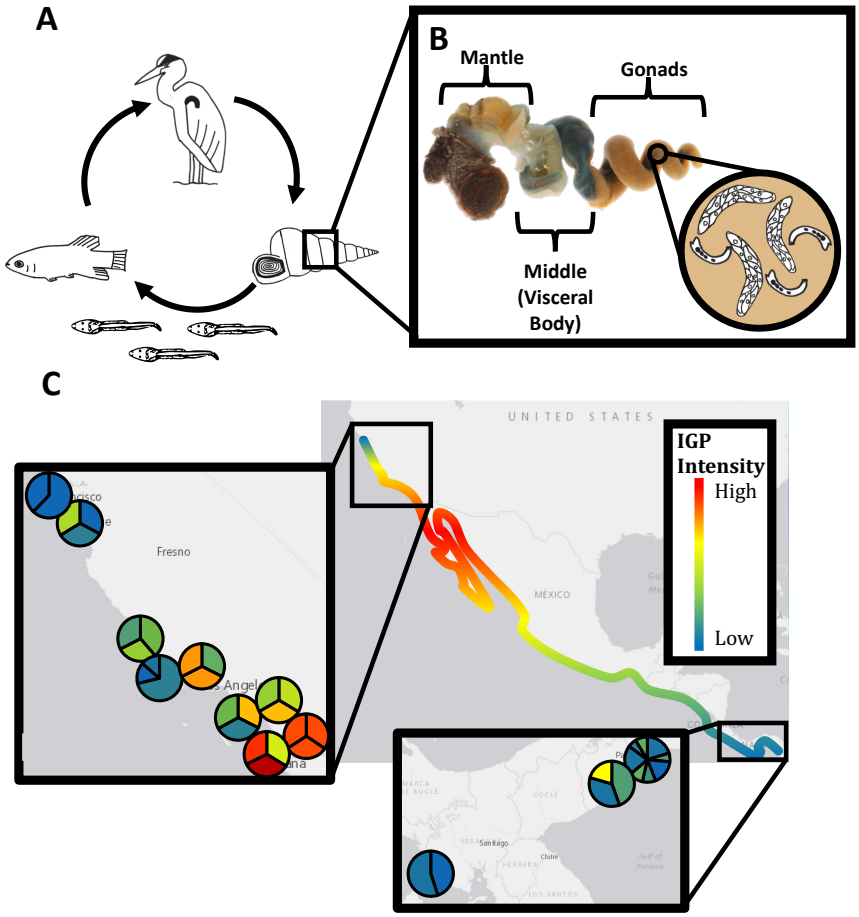

Figure 2.1 – Trematode life cycle, colony structure, and geographic variation in infection prevalence

A) Typical trematode complex life cycle. A single larva/egg infects a snail and colonially produces soldiers and reproductives (rediae), which castrate the snail, effectively stealing the body for their own reproduction (thus called "body-snatching"). Dispersive larvae (cercariae) produced by reproductives leave snails and encyst on/in secondary intermediate hosts, such as fish or mollusks. These cysts are then transmitted to shorebirds, their final host, through predation of the secondary intermediate host. Sexual trematode stages produce eggs which exit through the bird's feces and infect a new snail host. **B)** Diagram of a dissected *C. californica* snail. The gonadal region is the colony locus, where soldier and reproductives are produced. The middle and mantle regions are areas where invasion occurs, and soldiers are disproportionately represented there. Photo by Alex Wild. **C)** Theorized infection prevalence gradient from California to Panama (Torchin et al. 2015). Pies represent sampled estuaries and slice color represents site-level infection prevalence.

Table 2.1 – Trematode species name, initials, competitive rank (1 = most competitive), and dissected sample sizes.

Species Name	Initials	Competitive Rank	n
Parorchis acanthus	PARO	1	13
Himasthla rhigedana	HIMA	2	21
Himasthla sp. B	HIMB	3	29
Acanthoparyphium spinulosum	ACAN	4	37
Cloacitrema michaginensis	CLOA	4	10
Euhaplorchis californiensis	EUHA	5	58

Removing Outliers

A total of 7 outliers were removed. Outliers were determined to be those that were more than 2.5 interquartile range distance from the mean value for one of three metrics of interest: total reproductives, total soldiers, or proportion of soldiers outside gonad (POG).

Statistical Analyses

We used infection prevalence as our metric of IGP intensity, since they are highly correlated (Torchin et al. 2015) and colonies will even predate on other colonies of the same species (Hechinger et al. 2011). We calculated infection prevalence as the mean number of total trematode infections (All 20 species) per snail at both the estuary and site level (Table 2.4 and 2.5). We then compared estuary-level prevalence estimates with previous estimates from 2005 to show that IGP selection pressures can remain relatively constant over long time spans (Figure 2.2). This corroborates previous work showing long-term stability in infection prevalence across decades (Byers *et al.* 2016), and strongly suggests that trematode colonies should follow optimal caste ratio theory by investing differently in soldiers depending on the competitive environment.

We investigated three dependent variables for single-infection trematode colonies: total soldiers (i.e. soldier investment) and two metrics of soldier deployment: the proportion of soldiers in the mantle (PIM) and the proportion of soldiers outside the gonads (POG; Figure 2.1B).

We first describe the models used for total soldiers and then describe any differences for PIM models. For total soldiers, because our data was count data, we used generalized linear mixed models (GLMER) with a Poisson distribution, a log link function, and included site nested within estuary as our random variable to control for potential non-independence of trematode colonies. We used the BOBYQA optimizer and 100000 iterations to help convergence. We ensured model assumptions by examining overdispersion and examined residual plots using the DHARMa package (Hartig 2019). Our full model included snail size, total reproductives, species identity, total reproductive x species, site-level prevalence, estuary-level prevalence, and latitude. Both snail size and total reproductives are known to vary positively with total soldiers (Hechinger et al. 2011). Additionally, the total reproductives covariate functions as a proxy for colony growth and allows us to determine changes in relative allocation to soldier defense. Thus, a significant total reproductive x species interaction would indicate that species vary in how they allocate resources between defense and reproduction as the colony grows. Latitude, total reproductives, and snail size were z-score transformed to help models converge since variables were on very different scales. All continuous variables had low VIF scores (<4). We also included individual snail as an observation-level random effect to account for overdispersion in our model (Harrison 2014).

First, we determined which scale metric (site-level prevalence, estuary-level prevalence, or latitude) best explained total soldiers by comparing models with only one scale metric (but all other variables) to each other using AICc. Additionally, due to issues of model overfitting, only at this stage did we add prevalence x species. A significant prevalence x species interaction would indicate that species vary in the degree (slope) to which they increase soldier investment in response to increasing IGP intensity (hypothesis 3). Then we used that model to do backward selection and compared all models using AICc to find the absolute best fit model.

Soldier distribution was measured in two ways: PIM was log transformed to fit assumptions of normality, whereas POG was not transformed. To determine what factors influence soldier distribution, we used general linear mixed models (with a gaussian

distribution) similar to those used for soldier investment above. However, we did not include the interaction between total reproductives and species because of issues with overfitting and singularity. We removed the nested random effect of site within estuary for POG models because there was negligible variation between sites and estuaries, causing a singular fit of our model. Additionally, we added z-score transformed total soldiers to these models. We ensured model assumptions were met regarding data distributions by examining residual by predicted plots and normal quantile plots.

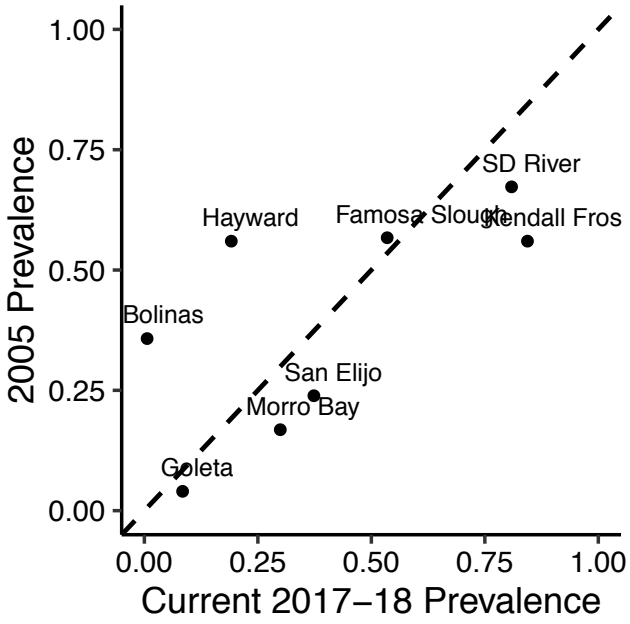

Figure 2.2 – A comparison of prevalence from 2005 and 2018.

Here we plot this study's California sample data from 2017/18 with previous estuary-level data from 2005. This relationship is weakly significant ($R^2 = 0.33$, $p = 0.07$). Dashed line is the 1:1 line.

RESULTS

The best fit model explaining total soldiers per colony included site-level prevalence (slope = 0.507 ± 0.229; rather than estuary-level prevalence or latitude), snail size, total reproductives, species identity, and a species identity*total reproductives interaction (Table 2.2; Figure 2.3). Adding an interaction between species identity and prevalence

did not improve our model (ΔAICc = -2.871; Table 2.3), suggesting that species do not respond differently to IGP intensity. The total number of soldiers was positively related to the total number of reproductives (Hechinger *et al.* 2011). In other words, as the colony grows it invests in both reproductives and soldiers. There was also a significant interaction between species and total reproductives that was primarily driven by *Euhaplorchis* (EUHA), which showed a significantly shallower slope than the other species, suggesting that EUHA invests comparably less in soldiers as their colony grows compared with the other, more dominant, species.

Our best fit model for the proportion of soldiers in the mantle (PIM) simply included species and total soldiers, but did not include prevalence or latitude (Table 2.2). This model was substantially better than all other models (ΔAICc ~ 10), suggesting that PIM is primarily affected by total soldiers in the colony (Table 2.3).

For the proportion of soldiers outside the gonad (POG), our best model included species, total soldiers, and latitude rather than prevalence (Table 2.2). This model was not substantially better than the model that removed total soldiers (ΔAICc = 0.205), removed total soldiers and latitude (ΔAICc = 1.359), or included total reproductives (ΔAICc = 1.266), but was substantially better (ΔAICc > 2) than models that included snail size or prevalence (Table 2.3).

Although support is weak, we did find that latitude positively correlated with the proportion of soldiers outside the gonad (POG). This work is the first to quantify variation in soldier distribution across space in trematodes and suggests that potentially at larger spatial scales, selection could be acting on trematodes to alter their soldier distribution. However, the effect of latitude was small and the could also be a function of unmeasured ecological differences, genetic drift, or adaptation, to name a few.

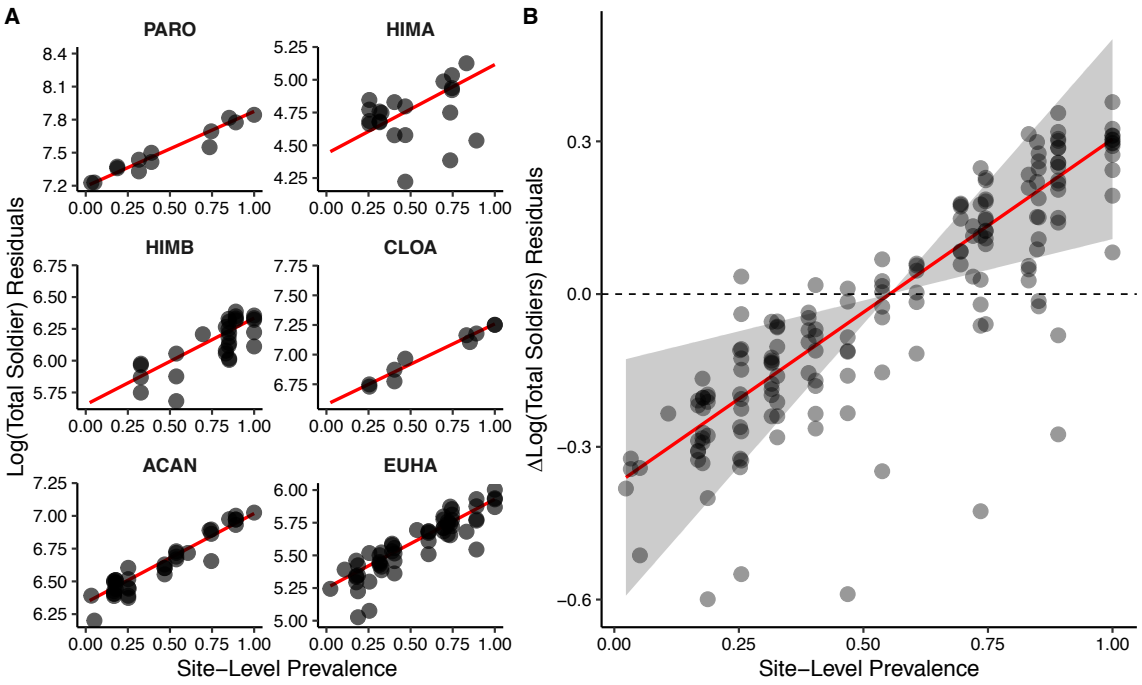

Figure 2.3 – Partial residual plots for the best fit model showing the effect of site-level infection prevalence on total soldiers.

A) Partial residual plots for each species. All species have the same slope (red line). Dots represent partial residuals of individual trematode colonies. B) Contrast plot showing how changes in site-level prevalence from the mean alter expected values of total soldiers along with confidence intervals.

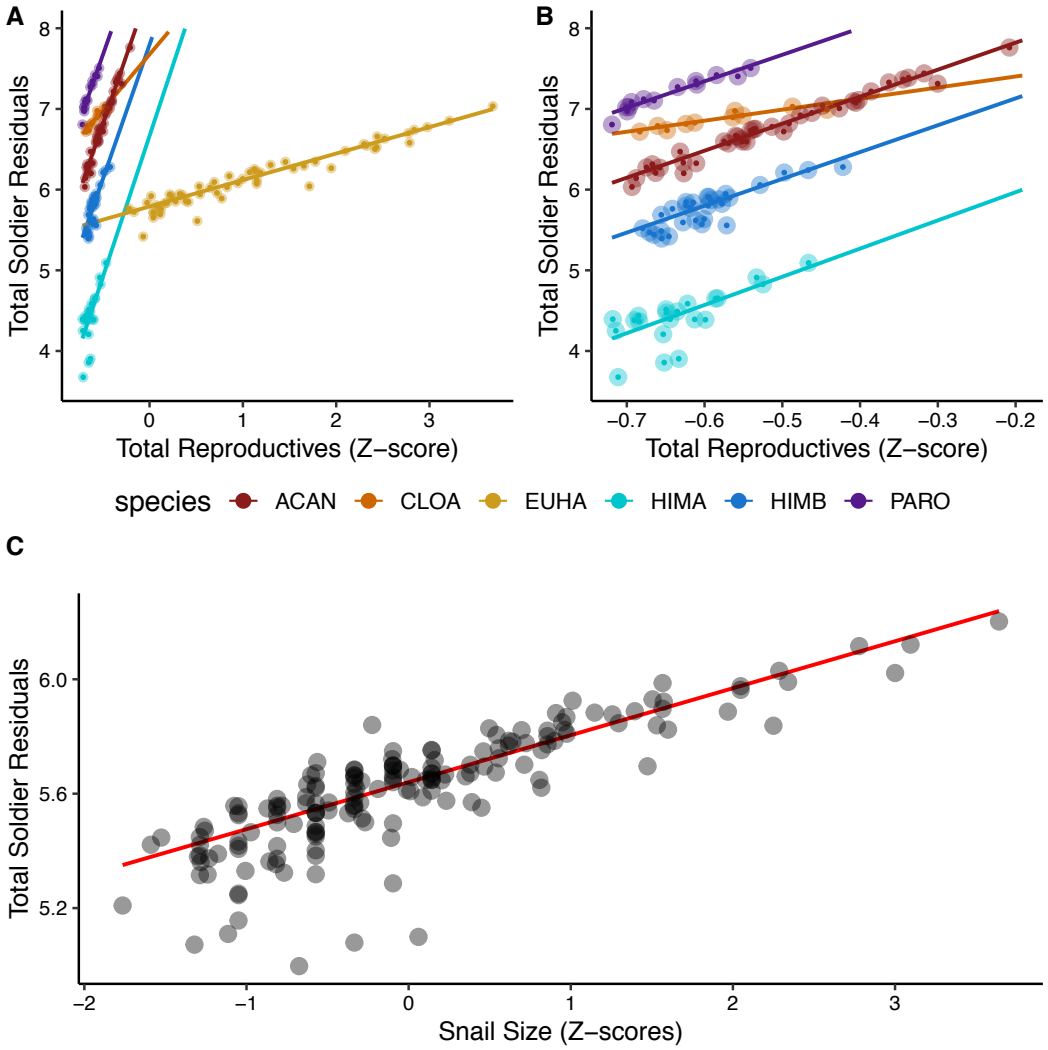

Figure 2.4 – Partial residual plots of covariates in best fit model of total soldiers.

A) The relationship between total soldiers and standardized total reproductives for all six species, controlling for other variables. Our best fit model displayed a significant interaction between species and total reproductives, meaning that there are significant differences in the slopes. Red = ACAN, orange = CLOA, yellow = EUHA, teal = HIMA, blue = HIMB, purple = PARO. B) This plot removes EUHA and allows a closer inspection of the slope differences between the five more dominant species. C) The relationship between standardized snail size and total soldiers controlling for other variables and using EUHA as the reference species to determine the y-intercept, because it is the most common species in our study. Our best fit model has a single slope for all species for the relationship between total soldiers and snail size.

Table 2.2 – Effect sizes and standard errors for variables included in best fit models. Dependent variables are total soldiers, proportion of soldiers in the mantle (PIM), and proportion of soldiers outside gonadal region (POG). Although species identity was significant for each best fit model, we do not include species here.

DV	Variable	Effect Size
Log(Total Soldiers) ~ Species + Total Reproductives + Total Reproductives x Species + Site-level Prevalence + (1|Estuary/site)		
	Snail Size	0.164 ± 0.061
	Prevalence Site	0.680 ± 0.224
	Total Reproductives x PARO	3.288 ± 2.783
	Total Reproductives x HIMA	3.494 ± 1.944
	Total Reproductives x HIMB	3.327 ± 1.949
	Total Reproductives x CLOA	3.256 ± 1.612
	Total Reproductives x ACAN	3.348 ± 0.866
	Total Reproductives x EUHA	0.328 ± 0.077
Log(PIM) ~ Species + Total Soldiers		
	Total Soldiers	-0.269 ± 0.064
POG ~ Species + Latitude + Total_Soldiers		
	Latitude	0.032 ± 0.015
	Total Soldiers	-0.025 ± 0.016

DISCUSSION

Our results demonstrate the first clear relationship between colony-level soldier investment and intraguild predation (measured as infection prevalence in this study) across a broad geographic range, supporting caste allocation theory (Oster and Wilson 1978). Additionally, we investigated this relationship across multiple spatial scales to determine the scale with which colonies respond. Consistent with our first hypothesis, we found a positive relationship between total soldier investment and intraguild predation (IGP) intensity, predominately at the site-level. Latitude did not predict soldier investment, providing more support that soldier investment is in fact responding to IGP intensity, rather than a metric that varies with latitude. Previous studies in trematodes and hymenoptera have examined how soldier investment varies geographically across

competitive environments (Wills et al. 2014, Lloyd and Poulin 2014), but have looked at too few localities to correlate these two variables. Previous laboratory work has shown that colonies can alter their soldier investment based on active coinfections (Lloyd and Poulin 2013a, MacLeod et al. 2018, Lagrue et al. 2018), but not based on IGP intensity exposure but not subsequent coinfection (Lloyd and Poulin 2014, Lagrue et al. 2018).

In contrast, our second prediction that a larger proportion of soldiers (i.e. higher deployment) should be found in the mantle of the snail (i.e. higher deployment) in estuaries with higher IGP intensity, was not supported. This may be because deployment is a fast process and colonies only alter deployment in response to active coinfection. Since we focused on single-species infections, we may not have seen this transition. One study in turtle ants found that soldiers only actively block the colony's nest entrance with direct enemy contact, but tend to spend the remaining time near the entrance (Powell et al 2017). Additionally, colonies demonstrated a bet-hedging strategy, decreasing the deployment of soldiers to new nests under threat of invasion, demonstrating that colonies can dynamically deploy soldiers based on interaction intensity (Powell et al 2017).

Finally, we did not find evidence that species differ in their response to IGP intensity for either soldier investment or deployment (hypothesis 3), as expected given competitive hierarchies. This suggests that changes in soldier allocation in response to intraguild predation do not explain the competitive rank of these species.

CONCLUSIONS

While previous work has shown that soldier investment varies between sites of high and low IGP intensity, here we demonstrate that soldier investment varies across a gradient of IGP intensity and quantify this effect. This work provides unique evidence from the field that supports caste allocation theory (Oster and Wilson 1978). Although caste allocation theory was derived for ants, no studies, to our knowledge, have looked at geographic variation in caste allocation in response across a gradient of interaction intensity. Our results demonstrate that trematode colonies can respond to their environment and are an excellent model system to understand caste allocation theory and

resource allocation in colonial organisms. However, it remains unclear if this relationship between IGP intensity and soldier investment is an inducible defense in response to an unknown cue (Lively 1986, Tollrian and Harvell 1999) or due to selection against low soldier investment. Reciprocal transplant experiments are needed to parcel out genotype x environment effects in this system.

Table 2.3 – Model selection for three dependent variables of interest.

Total soldiers, proportion of soldiers in the mantle (PIM), and proportion of soldiers outside gonads (POG). Df is the degrees of freedom, AICc is the corrected Aikake Information Criterion for each model. Delta is the difference between each model and our best fit model (highlighted in grey). Dashed grey lines indicate variables not used for a given dependent variable.

Total Repro	Species: Total Repro	Species	Snail Size	Latitude	Est Prev	Site Prev	Site Prev: Species	Total Soldiers	df	AICc	Delta	Model Lik	Model Wt
GLMM(Total Soldiers ~ Model + (1|Estuary/Site), family = "poisson")													
X	X	X	X			X			17	2396.514	0	1	0.648
X	X	X	X		X				17	2399.805	3.291	0.193	0.125
X	X	X	X				X		22	2401.038	4.524	0.104	0.068
X	X	X	X	X	X	X			19	2401.085	4.571	0.102	0.066
X	X	X	X						16	2401.657	5.143	0.076	0.05
X	X	X	X	X					17	2402.485	5.971	0.051	0.033
X	X	X							15	2404.752	8.237	0.016	0.011
X		X							10	2428.05	31.535	0	0
		X							9	2449.245	52.731	0	0
LMM(log(Prop Soldiers in Mantle) ~ Model + (1|Estuary/Site))													
		X						X	10	375.114	0	1	0.992
		X							9	384.93	9.815	0.007	0.007
		X	X						10	389.977	14.863	0.001	0.001
X		X	X						11	395.099	19.984	0	0
X		X	X		X				12	397.547	22.432	0	0
X		X	X			X			12	397.647	22.532	0	0
X		X	X	X					12	400.428	25.314	0	0
X		X	X	X	X	X			14	401.81	26.695	0	0
lm(Prop Soldiers Outside Gonads ~ Model)													
		X		X				X	9	-100.053	0	1	0.31
		X		X					8	-99.848	0.205	0.903	0.279
		X							7	-98.694	1.359	0.507	0.157
X		X		X				X	10	-98.497	1.557	0.459	0.142
X		X	X	X				X	11	-96.379	3.674	0.159	0.049
X		X	X					X	10	-94.808	5.245	0.073	0.022
X		X	X	X	X	X		X	13	-94.713	5.341	0.069	0.021
X		X	X		X			X	11	-93.261	6.793	0.033	0.01
X		X	X			X		X	11	-92.786	7.268	0.026	0.008

Table 2.4 – Summary statistics per estuary

Summary statistics per estuary of prevalence sample size, infection prevalence of individual species (ACAN:STIC), prevalence of all species combined (prev), abundance of trematode colonies of all snails sampled (abund), and richness (rich).

Estuary number	Estuary	Region	Variant	Latitude	Longitude	n	ACAN	AUST	CATA	CLOA	EUHA	HIMA	HIMB	INF
1	Santa Catalina	Panama	valida	7.635	-81.26	327	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000
2	Bique	Panama	mazatlantica	8.893	-79.657	739	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.004
2	Bique	Panama	valida	8.893	-79.657	448	0.067	0.000	0.000	0.000	0.002	0.000	0.000	0.000
3	Rio Venado	Panama	mazatlantica	8.895	-79.596	151	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3	Rio Venado	Panama	valida	8.895	-79.596	281	0.007	0.000	0.000	0.000	0.132	0.000	0.000	0.004
4	Famosa Slough	San Diego	californica	32.7518	-117.2288	301	0.003	0.000	0.000	0.000	0.346	0.007	0.003	0.013
5	SD River	San Diego	californica	32.759	-117.217	314	0.061	0.022	0.003	0.022	0.213	0.006	0.143	0.006
6	Kendall Frost	San Diego	californica	32.7912	-117.2306	314	0.048	0.003	0.000	0.013	0.191	0.006	0.054	0.010
7	San Elijo	San Diego	californica	33.012	-117.276	324	0.022	0.006	0.000	0.000	0.136	0.025	0.022	0.015
8	Carpinteria	Santa Barbara	californica	34.34	-119.533	314	0.166	0.000	0.000	0.016	0.054	0.083	0.146	0.016
9	Goleta	Santa Barbara	californica	34.418	-119.833	285	0.000	0.000	0.000	0.000	0.067	0.000	0.000	0.004
10	Morro Bay	Santa Barbara	californica	35.321	-120.847	344	0.000	0.000	0.000	0.006	0.093	0.032	0.003	0.006
11	Hayward	San Francisco	californica	37.6775	-122.162	329	0.033	0.000	0.003	0.003	0.000	0.033	0.000	0.006
12	Bolinas	San Francisco	californica	37.905	-122.651	331	0.003	0.000	0.000	0.000	0.003	0.000	0.000	0.000

Table 2.4 - Continued

Estuary number	MESO	PARO	PHOC	PROB	PYGI	REBU	RECE	REMA	RENB	RENC	RENIC	REPO	SMCY	SMMI	STIC	Prev	Abund	Rich
1	0.000	0.000	0.012	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.024	8	4
2	0.001	0.001	0.032	0.000	0.000	0.001	0.000	0.000	0.003	0.001	0.000	0.000	0.000	0.000	0.008	0.092	68	9
2	0.002	0.002	0.009	0.000	0.000	0.007	0.000	0.000	0.002	0.002	0.000	0.000	0.000	0.000	0.011	0.116	52	10
3	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.033	5	2
3	0.000	0.000	0.036	0.004	0.014	0.007	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.007	0.100	0.313	88	9
4	0.000	0.013	0.010	0.020	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.103	0.007	0.003	0.535	161	11
5	0.010	0.022	0.054	0.003	0.000	0.003	0.013	0.003	0.000	0.000	0.000	0.000	0.204	0.000	0.019	0.809	254	16
6	0.022	0.019	0.051	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.000	0.258	0.064	0.067	0.844	265	14
7	0.000	0.000	0.012	0.065	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.006	0.040	0.003	0.019	0.373	121	12
8	0.000	0.006	0.000	0.010	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.003	0.006	0.019	0.054	0.583	183	12
9	0.004	0.000	0.004	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.084	24	5
10	0.073	0.012	0.023	0.009	0.000	0.003	0.000	0.003	0.000	0.000	0.000	0.000	0.009	0.000	0.029	0.299	103	12
11	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.097	0.000	0.000	0.000	0.003	0.000	0.003	0.000	0.191	63	8
12	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	2	2

Table 2.5 – Summary statistics per site

Summary statistics per site of prevalence sample size, infection prevalence of individual species (ACAN:STIC), prevalence of all species combined (prev), abundance of trematode colonies of all snails sampled (abund), and richness (rich).

No	Estuary	site	Region	Variant	n	ACAN	AUST	CATA	CLOA	EUHA	HIMA	HIMB	INF	LGXI	MESO
1	Bique	1	Panama	mazatlantica	242	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.000
2	Bique	1B	Panama	mazatlantica	73	0.055	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3	Bique	2_inside	Panama	mazatlantica	208	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000
4	Bique	2_outside	Panama	mazatlantica	113	0.088	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000
5	Bique	2_outside	Panama	valida	121	0.215	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000
6	Bique	3	Panama	valida	253	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.004
7	Bique	3_bank	Panama	valida	74	0.000	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.027	0.000
8	Bique	4	Panama	mazatlantica	103	0.126	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.010
9	Rio Venado	1	Panama	valida	192	0.005	0.000	0.000	0.000	0.068	0.000	0.000	0.005	0.000	0.000
10	Rio Venado	2	Panama	mazatlantica	151	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
11	Rio Venado	3	Panama	valida	89	0.011	0.000	0.000	0.000	0.270	0.000	0.000	0.000	0.000	0.000
12	Santa Catalina	1	Panama	valida	147	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
13	Santa Catalina	2	Panama	valida	180	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000
14	Famosa Slough	1	San Diego	california	101	0.000	0.000	0.000	0.000	0.337	0.010	0.000	0.030	0.000	0.000
15	Famosa Slough	2	San Diego	california	100	0.000	0.000	0.000	0.000	0.600	0.010	0.000	0.000	0.000	0.000
16	Famosa Slough	3	San Diego	california	100	0.010	0.000	0.000	0.000	0.100	0.000	0.010	0.010	0.000	0.000
17	Kendall Frost	Alex	San Diego	california	108	0.028	0.000	0.000	0.009	0.194	0.000	0.046	0.000	0.000	0.046
18	Kendall Frost	Dan	San Diego	california	99	0.020	0.000	0.000	0.010	0.242	0.010	0.061	0.000	0.000	0.000
19	Kendall Frost	Emlyn	San Diego	california	107	0.093	0.009	0.000	0.019	0.140	0.009	0.056	0.028	0.000	0.019
20	San Elijo	31	San Diego	california	105	0.029	0.010	0.000	0.000	0.267	0.057	0.010	0.019	0.000	0.000
21	San Elijo	c13	San Diego	california	112	0.000	0.000	0.000	0.000	0.080	0.000	0.000	0.009	0.000	0.000
22	San Elijo	p21	San Diego	california	107	0.037	0.009	0.000	0.000	0.065	0.019	0.056	0.019	0.000	0.000
23	SD River	c10	San Diego	california	106	0.066	0.000	0.009	0.009	0.198	0.009	0.028	0.000	0.000	0.009
24	SD River	p16	San Diego	california	107	0.028	0.019	0.000	0.028	0.243	0.000	0.224	0.000	0.000	0.000
25	SD River	p22	San Diego	california	101	0.089	0.050	0.000	0.030	0.198	0.010	0.178	0.020	0.000	0.020
26	Bolinas	1	San Francisco	california	206	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 2.5 continued

27	Bolinas	2	San Francisco	california	125	0.008	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000
28	Hayward	1	San Francisco	california	108	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
29	Hayward	2	San Francisco	california	110	0.027	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
30	Hayward	3	San Francisco	california	111	0.072	0.000	0.009	0.009	0.000	0.099	0.000	0.018	0.000	0.000
31	Carpinteria	1	Santa Barbara	california	102	0.039	0.000	0.000	0.029	0.049	0.069	0.000	0.029	0.000	0.000
32	Carpinteria	2	Santa Barbara	california	110	0.382	0.000	0.000	0.018	0.073	0.145	0.000	0.000	0.000	0.000
33	Carpinteria	3	Santa Barbara	california	102	0.059	0.000	0.000	0.000	0.039	0.029	0.451	0.020	0.000	0.000
34	Goleta	1	Santa Barbara	california	203	0.000	0.000	0.000	0.000	0.089	0.000	0.000	0.000	0.000	0.005
35	Goleta	2	Santa Barbara	california	43	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.000
36	Goleta	3	Santa Barbara	california	39	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.000	0.000
37	Morro Bay	1	Santa Barbara	california	133	0.000	0.000	0.000	0.000	0.075	0.060	0.008	0.008	0.000	0.083
38	Morro Bay	2	Santa Barbara	california	99	0.000	0.000	0.000	0.020	0.081	0.030	0.000	0.010	0.000	0.101
39	Morro Bay	3	Santa Barbara	california	112	0.000	0.000	0.000	0.000	0.125	0.000	0.000	0.000	0.000	0.036

Table 2.5 continued

No	PARO	PHOC	PROB	PYGI	REBU	RECE	REMA	RENB	RENC	RENIC	REPO	SMCY	SMMI	STIC	prevalence	abundance	richness
1	0.004	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033	8	3
2	0.000	0.110	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.178	13	3
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.010	2	1
4	0.000	0.062	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.168	19	4
5	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.273	33	4
6	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.004	0.000	0.000	0.000	0.000	0.016	0.051	13	7
7	0.000	0.000	0.000	0.000	0.041	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.081	6	3
8	0.000	0.039	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.058	0.252	26	5
9	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.078	0.177	34	5
10	0.000	0.026	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.033	5	2
11	0.000	0.079	0.011	0.045	0.022	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.011	0.146	0.607	54	9
12	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.020	3	2
13	0.000	0.017	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.028	5	3
14	0.000	0.020	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.079	0.000	0.010	0.495	50	6
15	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100	0.000	0.000	0.720	72	4
16	0.040	0.000	0.050	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.130	0.020	0.000	0.390	39	8
17	0.019	0.056	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.278	0.037	0.130	0.852	92	11
18	0.000	0.040	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.000	0.323	0.010	0.051	0.848	84	11
19	0.037	0.056	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.178	0.140	0.019	0.832	89	14
20	0.000	0.038	0.162	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.057	0.000	0.048	0.695	73	9
21	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.009	0.116	13	4
22	0.000	0.000	0.028	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.009	0.065	0.009	0.000	0.327	35	10
23	0.009	0.019	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.142	0.000	0.028	0.538	57	12
24	0.037	0.093	0.000	0.000	0.009	0.028	0.009	0.000	0.000	0.000	0.000	0.271	0.000	0.009	1.000	107	12
25	0.020	0.050	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.198	0.000	0.020	0.891	90	12
26	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0	0
27	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	2	2
28	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019	2	1
29	0.000	0.000	0.000	0.000	0.009	0.000	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.082	9	3
30	0.000	0.000	0.000	0.000	0.018	0.000	0.225	0.000	0.000	0.000	0.009	0.000	0.009	0.000	0.468	52	8
31	0.000	0.000	0.010	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.010	0.000	0.255	26	8
32	0.009	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.018	0.073	0.745	82	9

Table 2.5 continued

33	0.010	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.029	0.088	0.735	75	8
34	0.000	0.005	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.108	22	5
35	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	1	1
36	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.026	1	0
37	0.015	0.008	0.023	0.000	0.008	0.000	0.008	0.000	0.000	0.000	0.000	0.015	0.000	0.008	0.316	42	11
38	0.000	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.081	0.404	40	7
39	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.188	21	4

Chapter 3: Soldier deployment varies over time and space

INTRODUCTION

Optimal caste allocation theory posits that selection should act on colonies such that colonies allocate resources to different castes (e.g. soldiers) or tasks (e.g. defense) to maximize life-time colony reproductive success (Oster and Wilson 1978). However, most studies (exceptions include Yang *et al.* 2004) have found that soldier reproduction is stable across populations and at the species-level (e.g. Huang and Wheeler, 2011; (Kaspari and Byrne 1995). Furthermore, studies have found that soldier production is not adjusted to local threat (i.e. competition) conditions within a population (e.g. Oster and Wilson 1978, Walker and Stamps 1986, Shibao 1999) with some notable exceptions (Passera et al. 1996, Aguilera-Olivares et al. 2017). This has led researchers to investigate whether constitutively-produced soldiers are dynamically deployed based on invasion threat. Here, we define deployment as the spatial allocation of soldiers across the snail body in response to shifting threats (Powell *et al.* 2017).

Most studies have focused on characterizing mean species-specific deployment of soldiers (e.g. Shibao 1998, Pike 2007) or deployment in response to immediate and direct competition or predation (e.g. Traniello 1981, Binder 1988). However, little work has looked at how colonies deploy soldiers in response to the competitive environment. One study found that turtle ants, which inhabit nesting cavities and add new nest cavities as the colony grows, followed a risk-limiting bet-hedging strategy, where under high threat conditions (i.e. in the presence of interspecific workers) fewer soldiers were allocated to new nest cavities (Powell *et al.* 2017). Here, we investigate colony changes in soldier deployment in response to natural variation in threat in the field.

Parasitic trematodes of the California horn snail, *Cerithideopsis californica,* are a tractable system to look at deployment in response to local threat. For a subset of these trematode species, a single diploid larvae (i.e. miracidium) or egg invades the host snail, castrates it, and subsequently divides asexually to form a colony generally within the

gonads of the snail (Galaktionov and Dobrovolskij 2013). For some species, this colony not only has large, reproductive larvae but also smaller, non-reproductive larvae that function as soldiers and use their mouthparts to ingest competing trematode species within a given snail host (Hechinger et al. 2011, Garcia-Vedrenne et al. 2016, 2017). These soldiers are produced in the gonads of the snail (where the colony resides) and are then deployed to the front of the snail, where new trematode infections initially invade (referred to hereafter as the invasion front; (Hechinger et al. 2011, Garcia-Vedrenne et al. 2016, 2017). These trematode parasites exhibit high intraguild predation (Kuris and Lafferty 1994; Kuris 1990, Sousa 1993, Lafferty et al. 1994), which has been shown to alter resource allocation (in terms of reproduction versus growth) in these colonies (Hechinger 2010). Additionally, it has been shown that the total soldier investment in colonies increases with intraguild predation (IGP) intensity, but that same study did not find evidence that soldier deployment varies with IGP intensity (see Ch. 2). Here, we experimentally test in the field whether trematode colonies dynamically deploy soldiers in response to local IGP intensity using a reciprocal transplant experiment.

We conduct a reciprocal transplant experiment using *Euhaplorchis californiensis* (EUHA) trematode colonies within Carpinteria Salt Marsh in southern California to investigate two predictions. First, we predict that EUHA colonies should dynamically deploy soldiers based on local IGP intensity, with low soldier deployment under low IGP intensity and high soldier deployment under high IGP intensity. Second, we predict that the relationship between total soldiers and IGP intensity is a phenotypically plastic response (rather than a constitutively produced defense). EUHA trematode colonies residing within the California horn snail are an excellent species to test these predictions because EUHA-infected snails are regionally common and can be identified non-invasively (e.g. Mordecai *et al.* 2016). For both of these predictions, we ask the following questions:

1) Are there colony differences in either soldier deployment or total soldier investment between high versus low IGP intensity sites before the experiment? Differences between sites are a prerequisite for investigating either of our

predictions, because changes between treatments would not be expected otherwise.

2) Are there overall colony differences before and after the experiment? This would suggest that a variety of other variables, such as seasonality, could be affecting soldier deployment or total soldier investment.
3) Are there colony differences in soldier deployment or total soldier investment between destination locations? This would indicate that colonies respond to IGP threat dynamically for deployment or plastically for soldier investment.

METHODS

Collection

Cerithideopsis californica, California horn snails, were collected from two channels in Carpinteria Salt Marsh (CSM), California, USA (34.40° N, 119.53° W; Figure 3.1). The northern channel has had low trematode infection prevalence (e.g. Hechinger and Lafferty 2005), and the southern channel has had high infection prevalence, based on surveys from 2012-2013 (Hechinger et al. 2017; Figure 3.1). The channels were approximately 550 m apart. We collected 726 snails from the northern channel and 1248 snails from the southern channel between July 15th and July 19th of 2018. The time frame of the experiment was chosen specifically to coincide with high bird densities and high periods of trematode invasion based on previous surveys at Carpinteria that noted immature trematode infections within snails (Hechinger et al. 2017; Lafferty 2001; Figure 3.2). We chose this time in order to maximize the potential impact of IGP on colonies.

Prevalence estimates and snail shedding

We dissected one hundred snails from each site to estimate richness, prevalence of individual trematode species, and overall infection prevalence (all trematode species combined). In the laboratory, snails were destructively processed following Torchin et al (2005), and trematode species within a snail were identified following Martin (1972) and R.F. Hechinger and T. C. Huspeni (unpublished manuscript). Overall, infection

prevalence was used as a proxy for intra-guild predation threat as the two have been shown to highly correlate (Torchin *et al.* 2015).

To obtain snails infected by *Euhaplorchis californiensis* (EUHA) for the experiment, we non-destructively screened the remaining snails for infection following standard techniques (e.g. Mordecai *et al.* 2016). We placed snails from the >20mm size class from each site (low or high prevalence) into individual wells (12-well and 6-well plates) of filtered seawater and heated them to 25-30°C for 30 minutes under halogen bulbs. Snails were then maintained under fluorescent lights for >2 hours. We then screened individual wells for EUHA cercariae (larval stage that reproductive trematodes produce), which indicated that these snails had been infected. The remainder of the snails that were not EUHA-infected were released back to their original collection locations. We found over one hundred EUHA-infected snails at each site. Fifteen EUHA-infected snails from each site were also fully dissected to determine if there were initial differences in either EUHA soldier investment or deployment.

Experimental Setup

Undissected EUHA-infected snails (80 from each site) were then cleaned, spray-painted a unique treatment color (yellow = high, green = low). At each site (low and high prevalence), we placed eight 30-cm diameter cages (see Buck *et al.* 2017) along the channel, partly submerged so that snails were subject to the effects of the tide and to minimize the risk of either drowning or desiccation (Figure 3.3). In each cage, we placed 5 snails from each original site (high or low prevalence) for a total of 10 snails per cage. We used this relatively low density per cage in order to maximize the probability of infection by minimizing safety in numbers (Buck et al. 2017). This gave us n=40 for each of our four treatments (High to High, High to Low, Low to Low, Low to High). Experimental cages were then left out in the field for 4.5 months (July 21st, 2018 – November 30th, 2018), which corresponded with the bird migration, and high prevalence of coinfections (Hechinger et al. 2017; Figure 3.2).

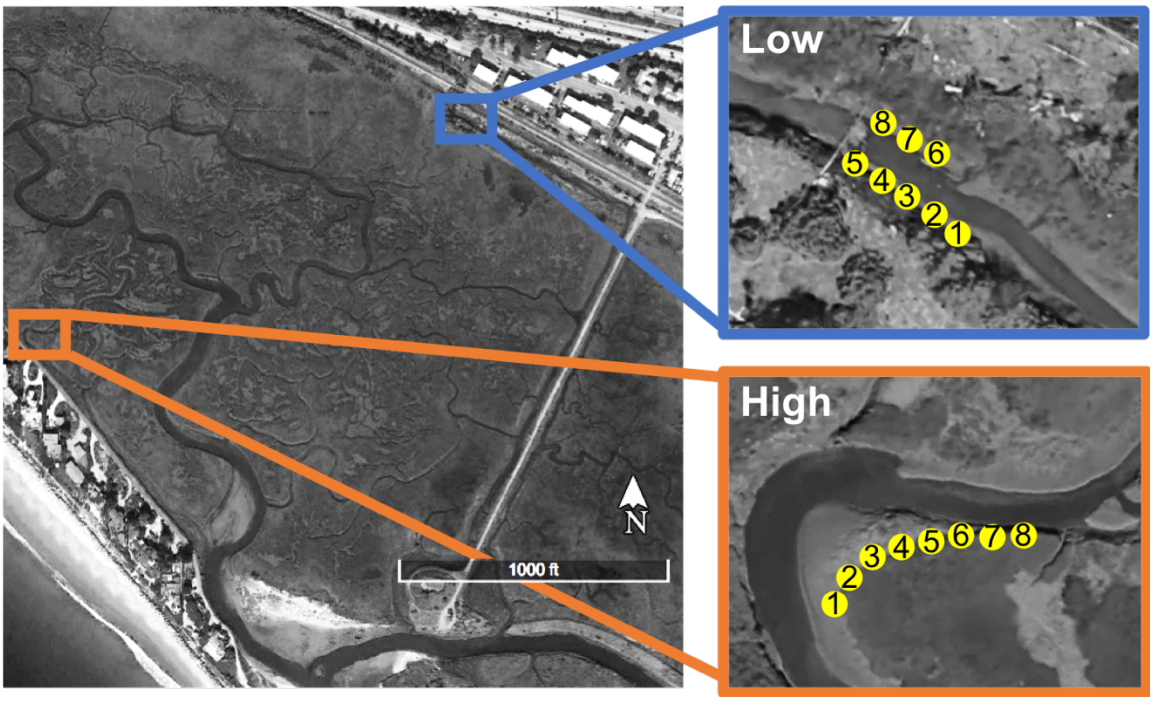

Figure 3.1 – Map of sites at Carpinteria Salt Marsh.

Sites within Carpinteria Salt Marsh were chosen that had either high prevalence (orange square) or low prevalence of trematode infections (blue square). Yellow circles and numbers represent the location of individual cages within each site for our reciprocal transplant experiment.

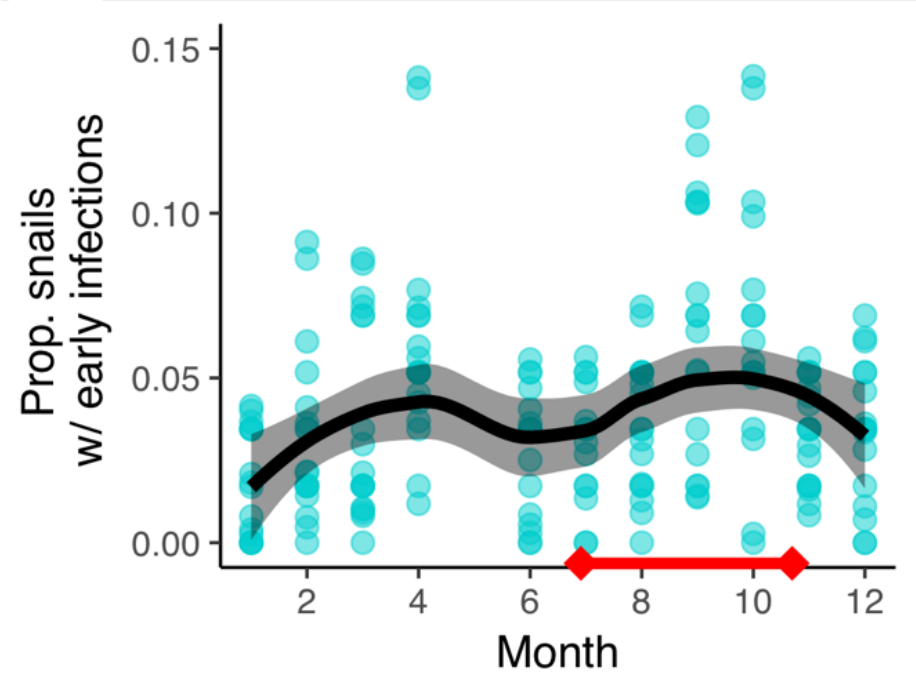

Figure 3.2 – The proportion of snails with immature infections across time.

The proportion of immature infections across time based on previous data from Carpinteria Salt Marsh (Hechinger et al. 2017). Immature infections are those that do not have cercariae yet or have very few cercaria and take up only a subset of area that a mature colony would. Many immature infections indicate a time of high IGP intensity. The duration of the experiment covers the time of high bird prevalence and high immature infection rate. Black line and grey area represent the mean and standard error for the proportion of snails with immature infections across sites. The red line marks experimental duration, which matches the time of highest immature infections.

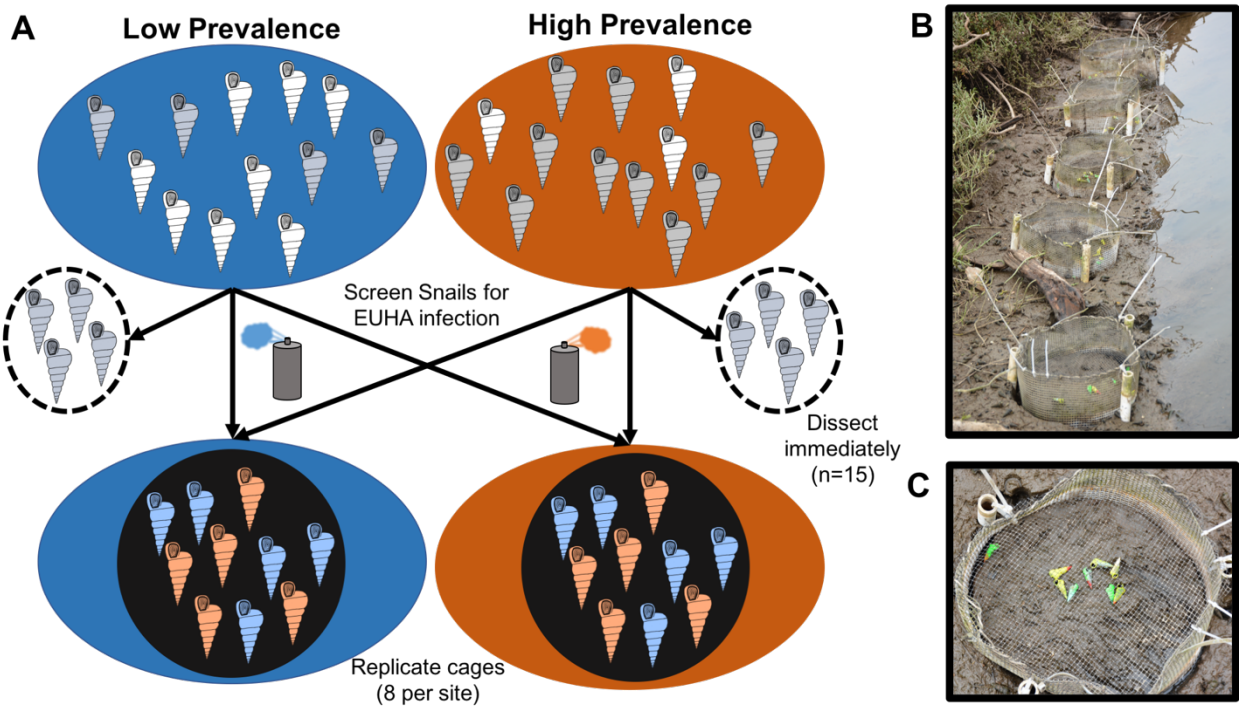

Figure 3.3 – Reciprocal transplant experimental design.

A) Schematic of experimental design (Top to bottom). White (uninfected) and grey (infected) snails are collected from high and low infection prevalence sites at Carpinteria Salt Marsh. Snails were non-invasively screened for *Euhaplorchis* (EUHA) infections. A subset of EUHA-infected snails (grey) were dissected before the experiment (stippled line). The remaining snails (80 from each site) were then spray painted (blue = low infection, orange = high infection) and 5 snails from each site were placed in replicate cages either at the high prevalence site or low prevalence site. There were 8 cages per site. Black circles represent a cage at high and low prevalence sites and the color of the snails represent the source location of EUHA-infected snails. B) A picture of cages partially submerged in a channel out in the field. C) Ten snails (5 from each site) were placed out in the field in cages.

Recollecting experimental snails

Due to a storm near the end of the experiment, cages in our high IGP threat site (i.e. in the northern channel) were damaged. Sediment had partially submerged cages 1-4, though the snails were still at the top of the sediment and able to move. The sediment had shifted under cage 5, such that the bottom of the cage was not submerged and all snails were lost. Cage 6 was completely lost and not recovered. All remaining cages were in good condition. For snails that were recaptured after the experiment, mortality rates were higher in the high prevalence site than the low prevalence site, likely due to storm effects (14/49 vs 14/76; Table 3.1).

Table 3.1 – Status of snails post-experiment

High and low transplant sites varied in the number of snails recovered. Of the snails recovered, snails were catalogued as dead, infected with a non-EUHA trematode species, coinfected with EUHA and another trematode, or infected with only EUHA.

Transplant Site	Snail state	n
High	EUHA infection	31
	Non-EUHA infection	3
	Coinfection with EUHA	1
	Not Recovered	31
	Snail Dead	14
Low	EUHA infection	56
	Non-EUHA infection	0
	Coinfection with EUHA	6
	Not Recovered	4
	Snail Dead	14

Dissection

Before the experiment, a total of 15 snails from each site (high and low) were dissected to give a baseline value for differences in soldier deployment and soldier investment between the sites. After the experiment, 17 EUHA-infected snails were dissected from

each treatment, except for the high prevalence source to high prevalence destination treatment (n=14). We assessed caste allocation and distribution for snails that had single-species infections with fully-formed cercariae present (indicating a mature colony).

To determine the deployment of soldiers across the snail body, snails were divided into three sections: the mantle, the middle (visceral body), and the gonadal region (Hechinger *et al.* 2011). Because of the extremely high numbers and small size of colonial members (reproductives and soldiers), only a proportion of the gonadal region was counted and total soldiers and reproductives were estimated. For the mantle and middle, each region was teased apart using forceps, releasing soldiers and reproductive trematodes into a Syracuse watch glass for ease of counting. Seawater was added to the dish and larger clumps were teased apart or squashed between plates of glass and counted separately. Additionally, soldiers and reproductives that dislodged from a snail body during dissection and pooled in the bottom of the dissection watchglass were also counted.

Statistical analyses

Are there colony differences between source sites before the experiment?

To answer this question, we compared snails dissected before the experiment from high and low prevalence sites. We used a Poisson generalized linear model with total soldiers as the dependent variable (DV), and total reproductive trematodes and snail size Z-scores as covariates. Because of overdispersion, we added a random effect of individual snail host for our total soldier model only (Harrison 2014). We also included source (high vs low IGP intensity) as an independent variable.

For the proportion of soldiers in mantle (PIM) and the proportion of soldiers outside gonads (POG), we used a linear model and did not transform the data because they fit model assumptions. *Note, we did not include total reproductives in these models, as previous work (Chapter 2) found this variable to be insignificant.

DV (before the experiment) ~ Source + Total Reproductives* + Snail Size (1)

Are there colony differences before and after the experiment?

For this question, we compared colonies before the experiment with colonies after the experiment that were transplanted back to their source location (Pre_Post; high to high or low to low). This allowed us to remove the potential effect of treatment and to simply see if there was a difference between snail colonies at the two time points, due to possible seasonal effects or cage effects. We also included total reproductives and snail size Z-scores as covariates for our model with total soldiers as the dependent variable (DV) but only included snail size for models with POG or PIM as the dependent variable.

DV ~ Pre_Post + Total Reproductives + Snail Size (2)

Are there differences between the effects of the treatments?

For this, first we looked only at colonies dissected after the experiment. We included the destination (high or low), the source location (high or low), and an interaction between them. We also included total reproductives and snail size Z-scores as covariates and cage number as a random effect. For PIM and POG we did not include total reproductives as a covariate.

DV ~ Source * Destination + Total Reproductives + Snail Size + (1|Cage) (3)

Additionally, we looked to see if there were differences between treatments in the *change* from their source before the experiment. In order to do this, we calculated the mean values for our various dependent variables (total soldiers, PIM, POG) for each site BEFORE the experiment. We then subtracted this value from each experimental snail colony based on their source location. This gave us a delta for each snail colony. To compare these deltas, we used the same model as above, but used delta values for the dependent variables:

ΔDV ~ Source * Destination + Total Reproductives + Snail Size + (1|Cage) (3B)

RESULTS

There was a 3-fold difference in infection prevalence between high and low sites (Table 3.2)

Table 3.2 – Differences in parasite richness and infection prevalence between two sites. The number of snails (out of 100 per site) that were uninfected or infected with a particular trematode species (ordered based on decreasing competitive rank). Infection prevalence was calculated as the proportion of snails infected. Richness was the total number of trematode species per 100 snails.

		Site	
Species	Initials	High	Low
Austrobilharzia sp	AUST	1	0
Parorchis acanthus	PARO	3	1
Himasthla rhigedana	HIMA	19	0
Himasthla sp. B	HIMB	2	1
Acanthoparyphium spinulosum	ACAN	3	0
Cloacitrema michaginensis	CLOA	3	0
Euhaplorchis californiensis	EUHA	34	19
Phocitremoides ovale	PHOC	2	0
Stictodora hancocki	STIC	1	0
Small microphallid	SMMI	0	1
	Uninfected	32	78
	n	100	100
	Prevalence	0.68	0.22
	Richness	9	4

Are there colony differences between source sites before the experiment?

Before the experiment, there were no differences between colonies from high and lowprevalence sites in terms of total soldiers ($\chi^2 = 0.4286$, df = 1, p-value = 0.5126). Although total reproductive trematodes did not explain soldier investment ($\chi^2 = 0.475$, df = 1, p-value = 0.4907), snail size did ($\chi^2 = 4.179$, df = 1, p-value < 0.0409). Neither source (high or low; F-value = 0.5278, df = 1, p-value = 0.4740) nor snail size (F-value = 0.1750, df = 1, p-value = 0.6791) explained the proportion of snails in the mantle (PIM)

for colonies dissected before the experiment. There was, however, a nearly significant difference in the proportion of soldiers outside the gonads (POG) between high and low prevalence source sites (F-value = 3.9877, df = 1, p-value = 0.0564), with high prevalence sites having a higher POG than low prevalence sites (Fig 3.4A). Snail size did not significantly explain POG (F-value = 0.0123, df = 1, p-value = 0.9127).

Are there colony differences before and after the experiment?

Colonies did not differ in their total soldier investment before and after the experiment (χ^2 = 0.9271, df = 1, p-value = 0.3356). However, the proportion of soldiers outside the gonads and the proportion of soldiers in the mantle (POG and PIM) decreased during the experiment, though this trend was non-significant for POG (F-value = 2.596, df = 1, p-value = 0.1126; F-value = 7.4535, df = 1, p-value < 0.01; Fig 3.4B).

Are there differences between the effects of the treatments?

Next, we looked at only our experimental data to see if there was an effect of treatment. There were no significant effects of source, destination, or their interaction for total soldiers, PIM, or POG. There was a marginally significant effect of source (χ^2 = 3.502, df = 1, p-value = 0.0613), but not an effect of destination (χ^2 = 0.0093, df = 1, p-value = 0.9231) or their interaction (χ^2 = 0.0371, df = 1, p-value = 0.8474) on proportion of soldiers in the mantle (PIM).

In terms of change, colonies originally from the high infection site decreased their PIM more than colonies from the low infection site (Figure 3.4C). Neither change in total soldiers nor change in POG were significantly explained by source, destination, or their interaction, but there was a non-significant effect of source for change in POG (χ^2 = 1.9899, df = 1, p-value = 0.1583), with colonies from the high prevalence site decreasing their POG while colonies from the low prevalence site did not change (Figure 3.4C).

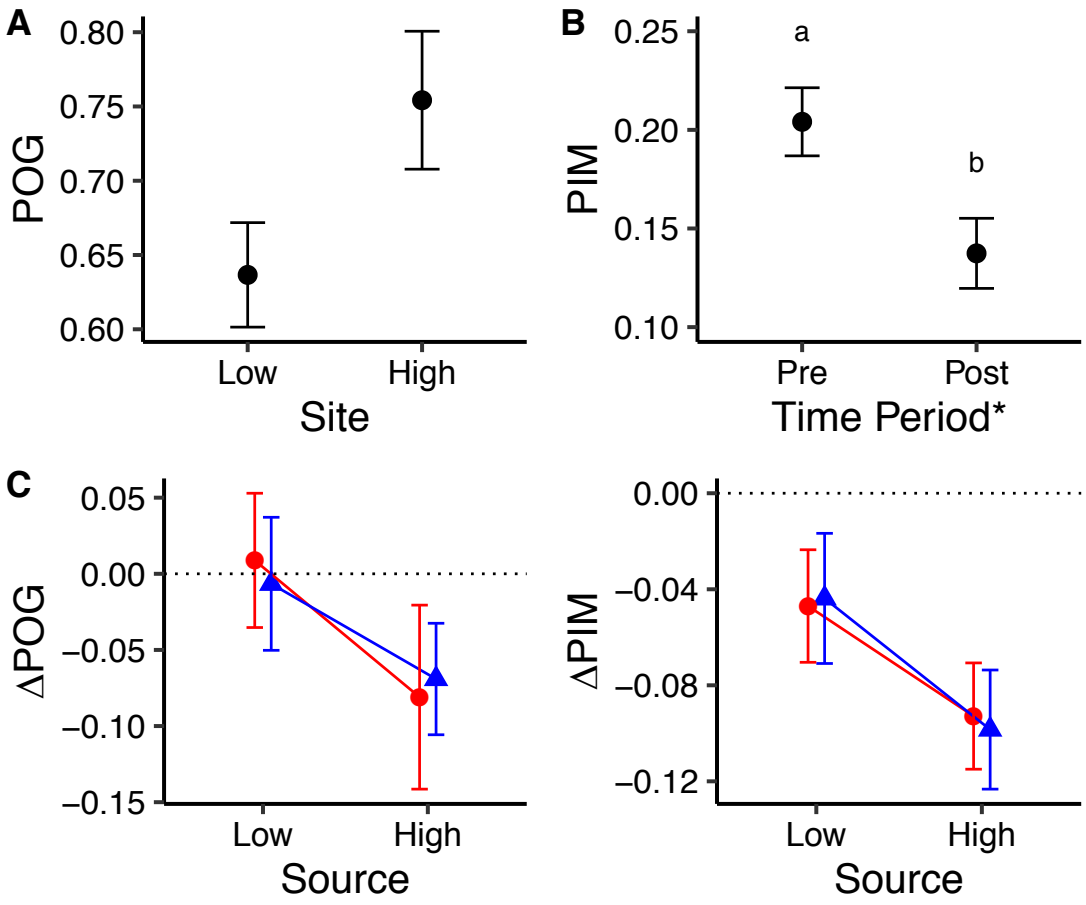

Figure 3.4 – Results from reciprocal transplant experiment

A) There was a nearly significant difference in the proportion of soldiers outside the gonads (POG) between sites before the experiment (p-value = 0.0564). B) The proportion of soldiers in the mantle (PIM) was significantly lower after the experiment than before the experiment (p-value < 0.01). C) The effect of source and destination on soldier deployment: ΔPOG and ΔPIM. Red circles and blue triangles represent colonies transplanted to high and low prevalence areas respectively. Colonies originally from the high prevalence site decreased POG, whereas colonies from the low infection site did not decrease POG (p-value = 0.158). Colonies originally from the high prevalence site decreased PIM more than colonies from the low prevalence site (p-value = 0.0613). The dotted line represents no change.

DISCUSSION

In this study, we investigated whether or not colonies can plastically respond to their competitive environment in terms of soldier investment and soldier deployment. We found that colonies from our high infection prevalence site had a higher proportion of their soldiers outside the gonads (POG) than colonies from the low prevalence site (Figure 3.4A), suggesting that colonies may deploy more soldiers under conditions of higher threat. This contradicts our survey findings (Ch 2), that did not find a relationship between deployment (either PIM or POG) and infection prevalence across a large geographic range from northern California to Panama. This is the first evidence, to our knowledge, that reveals local between-site differences in soldier deployment in social trematodes. Given the few number of sites, we cannot say that this is a response to differences in infection prevalence (and intraguild predation intensity) alone, as opposed to other unmeasured between-site environmental differences. However, infection prevalence can be extremely patchy and can change dynamically within an estuary (e.g. (Lafferty *et al.* 1994), suggesting that the ability to respond quickly to changing threat conditions through deployment would be beneficial.

There was a significant difference in soldier distribution before and after the experiment. Importantly, because we destructively sample snails to determine soldier investment and soldier distribution, we are not actually comparing the same colonies before and after the experiment. Nevertheless, the colonies dissected after the experiment, including only those that remained at their source location, had significantly lower proportion of soldiers in the mantle (PIM) than colonies dissected before the experiment Figure 3.4B). This change was primarily driven by colonies from the high IGP intensity site which decreased their PIM during the experiment such that PIM was not significantly different between the two sites after the experiment (Figure 3.4C). It did not matter whether or not the sites were transplanted home or away. These results suggest that colonies may alter the distribution of soldiers across the snail body over time, but not necessarily in response to IGP threat. One possibility is that colonies alter their soldier distribution seasonally. This could align with bird migration, which itself aligns well with

the proportion of early coinfections. Bird migration peaks in late fall/winter annually (Lafferty 2001) and therefore trematode colonies could be responding to seasonal changes that indicate bird migration, and by extension, high competition for snail hosts (Hechinger and Lafferty 2005). However, in this case, we would expect soldier deployment to increase during the experiment, rather than decrease as we found.

In our reciprocal transplant experiment, there was no effect of destination on soldier deployment (Figure 3.4C). This suggests that colonies do not dynamically deploy soldiers in response to threat, or that our experiment was too short for such deployment to develop. On the other hand, there was an effect of source on soldier deployment, such that deployment to the mantle (PIM) decreased more from high infection prevalence sites than low infection prevalence sites, erasing any differences in deployment between sources. These results suggest that environmental differences between the two sites may be stronger in July than they are in December, such that high infection sites increase deployment.

Originally, this experiment sought to test whether differences in soldier investment in response to infection prevalence were due to phenotypic plasticity or local adaptation. However, we did not find any difference in soldier investment between our sites, and therefore we would not expect to see a change in soldier investment based on transplantation and could not actually test whether colonies plastically alter soldier investment in response to IGP threat (i.e. infection prevalence). This is not wholly incongruent with previous survey findings, which found that sites that varied in infection prevalence within an estuary did not always vary similarly in soldier investment (see Ch 2). One mechanism for this could be that other stronger signals, such as total reproductives or snail size, might swamp out the effects of infection prevalence. Additionally, colonies in starving hosts may allocate fewer resources to soldiers, although this has not been demonstrated in single-species infections (Lloyd and Poulin 2013a, Mouritsen and Andersen 2017). If this is the case, lower patch quality at our high infection site, especially if exacerbated in the fall (during our experiment) may minimize differences in soldier investment in response to infection prevalence.

Overall, we show that soldier deployment varies spatially and temporally, although we could not determine whether this variation was due to dynamic deployment in response to environmental factors such as seasonality, bird density, or infection prevalence. Clearly more work is needed to assess under what conditions colonies alter their soldier deployment. Future directions should include monthly surveys to illuminate seasonal changes in soldier deployment or investment and determine what environmental factors colonies could be responding to. Additionally, future reciprocal transplant experiments should cover multiple pairs of high and low infection prevalence sites within a given estuary, each with higher sample sizes, in order to account for variation in other factors between sites, such as growth rate or patch quality. Altogether, this work provides novel insights into how colonies respond to environmental changes, and provides fodder for future experimental work in this system.

Ch 4: Caste ratio and soldier distribution varies between trematode species of the California horn snail

INTRODUCTION

Competition between species is a fundamental force mediating coexistence and ecological community composition (e.g. Gause 1934, Tilman 1982). Competing species can coexist within a landscape by offsetting differences in competitive ability with niche differences, such as resource use or predation risk (Levin 1970, Chesson 2000). Life-history tradeoffs can especially promote coexistence between species by minimizing differences in competitive abilities and increasing niche differences (Kneitel and Chase 2004, Edwards and Stachowicz 2010, 2011). One type of life-history tradeoff commonly studied is the colonization-competition tradeoff (Levins 1969, Hastings 1980, Nee and May 1992, Tilman 1994) also called the dominance-discovery tradeoff (Fellers 1987). In this case, there is a tradeoff between competitive ability and colonization rate such that competitively inferior species colonize open habitat first but are later displaced by later-arriving superior competitors.

Extensive work has focused on understanding the mechanisms that promote coexistence in linear dominance hierarchies (e.g. Stanton et al. 2002, Adler et al. 2007a), which are common in social insects, such as ants (e.g. Wilson and Southwood 1990, Vepsäläinen et al. 2000, Palmer et al. 2000) where competition is nearly ubiquitous (Hölldobler and Wilson 1990). Comparatively less work has been done focusing on the specific mechanisms that support these linear dominance hierarchies (e.g. Palmer 2004). However, ecological and economic impacts of invasive eusocial species, such as fire ants, *Solenopsis invicta*, and Argentine ants, *Linepithema humile*, have drawn attention to the factors underlying dominance (e.g. Holway *et al.* 1998). Numerical differences between colonies can be a strong indicator of competitive dominance for territory/nest sites (Adams 1990; Holway et al. 1998, Holway and Case 2001). For instance, the dominance hierarchy in a guild of acacia ants was solely explained by colony size, rather than worker size or deployment speed, with the dominance hierarchy reversing when colony size was reversed (Palmer 2004). Additionally, this study found that these species fought primarily

in one-on-one combats where mortality between species pairs was equal, further emphasizing the importance of colony size over all else (Palmer 2004).

Similarly, in order to predict outcomes of direct fights between ant colonies, Franks and Partridge (1994) applied Lanchester's theory of combat (1916). Lanchester's theory of combat are mathematical models originally designed for military combat to predict the outcome of battles based on army size and the efficacy of individual fighting units (Lanchester 1916). Lanchester's square law predict that organisms fighting in open fields should invest more in many, small soldiers that can attack as a group. However, when fights are one-on-one, such as the acacia ants described above, Lanchester's linear law predicts that it is better to invest in fewer, large soldiers (Wills *et al.* 2018). This has been experimentally tested in ants (McGlynn 2000), where the fighting arena was manipulated. McGlynn (2000) demonstrated that large ants were behaviorally dominant under one-on-one competition and small ants were behaviorally dominant in more open combat. Despite the power of Lanchester's theory of combat, this theory has not been applied to explain competitive dominance hierarchies like the social trematodes residing in California horn snails.

Host-parasite system

Roughly 20 species of trematode utilize the California horn snail as their first intermediate host (Kuris 1990, Sousa 1993). All species of interest infect the gonads, such that there are no clear niche differences between these species (Mordecai *et al.* 2016). Within a single snail, only one species of trematode (originating from a single clone) can exist due to high intraguild predation that follows a well-understood and relatively strict competitive hierarchy, such that competition is asymmetric: superior competitors can successfully invade snails infected by inferior competitors and displace them, while inferior competitors can only infect uninfected snails or snails infected by more inferior competitors (Kuris 1990, Huspeni 2000). This competitive hierarchy is primarily based on direct evidence of infection succession from the field (Kuris 1990, Huspeni 2000). Since snails almost never purge an infection, and therefore any transition

between two trematode species is due to direct competition and not to elimination by the host (Kuris 1990, Sousa 1993, Kuris and Lafferty 1994, Lafferty et al. 1994). Trematode species whose colonial stage have mouthparts are the species that are competitively superior to trematode species whose colonial stages do not have mouthparts (Kuris 1990, Sousa 1993, Hechinger 2010, Mordecai et al. 2016). Additionally, these species (with the exception of *Catatropis johnstoni*) have a caste division of labor, with colonies composed of reproductives and smaller, non-reproductive soldiers that function to fight off other trematodes (Hechinger et al. 2011, Garcia-Vedrenne et al. 2016, 2017). These soldiers are produced in the gonads of the snail, where the colony locus is, and then move to the front of the snail, where coinfections from other trematodes species generally occur (Hechinger et al. 2011). Finally, modelling work has suggested that half of the local trematode diversity in this system can be explained by a colonization-competition tradeoff between these species, where more dominant species colonize at lower rates than subordinate species (Mordecai *et al.* 2016). In this way, dominance is akin to 'behavioral dominance' (Schoener 1983) rather than ecological dominance (Paine 1966, 1969).

Although the competitive dominance hierarchy of trematodes is well understood in the California horn snail, the specific mechanisms generating and maintaining this hierarchy are poorly understood. In general, this competitive hierarchy should depend on both how well species can invade hosts and outcompete others, and how well species can defend their colony from such invasions and thus prevent replacement by another species. Correlational evidence between reproductive rediae (those species with mouthparts) suggests that larger rediae (with larger mouthparts) should be dominant (Kuris 1990). However, since the discovery that these trematodes have a soldier caste, no work has systematically compared soldier morphology or colony composition between competing species.

In this study, we investigate the drivers of a classic competitive dominance hierarchy in social trematodes residing in the California horn snail. Specifically, we utilize differences between species, in terms of individual soldier attributes, as well as colony level attributes, to quantify factors responsible for the observed dominance

hierarchy. Finally, we combine these metrics to calculate Lanchester's laws for each species to assess how well colonies from different species can defend themselves against invaders. We ask three questions and make corresponding predictions:

1) Does differential allocation between defense and reproduction explain competitive rank? If absolute defense allocation confers increased protection for a colony, then species that allocate more resources to defense (either total biomass or soldier number) should have higher competitive rank.
2) Does soldier deployment explain competitive rank? If the proportion of soldiers deployed to the front of the snail (i.e. invasion front) relates to the readiness of a colony to fight off coinfection, then we would expect species with a higher proportion of soldiers in the mantle to be more dominant.
3) Do estimates of colony fighting ability explain the competitive dominance hierarchy? If defensive colony fighting ability (as defined by Lanchester's laws) is more important than invasion ability for structuring the competitive dominance hierarchy, we would expect to find a positive relationship between colony fighting ability and dominance hierarchy.

METHODS AND MATERIALS

Collection

We focused on six trematode species that the infect California horn snail, *Cerithideopsis californica*: *Parorchis acanthus* (PARO), *Himasthla rhigedana* (HIMA), *Himasthla* sp. B (HIMB), *Cloacitrema michaginensis* (CLOA), *Acanthoparyphium spinulosum* (ACAN), and *Euhaplorchis californiensis* (EUHA). These species were chosen because they possess a caste division of labor, vary in their competitive rank, and span across three families (Figure 4.1; Hechinger et al. 2009, Mordecai et al. 2016). California horn snails were collected from nine estuaries across California: San Diego (4), Santa Barbara (3), and San Francisco (2). In Panama, we collected California horn snails from 5 locations, but we only found infections in 3 of those locations. We collected trematode colonies from this broad geographic range to get species-specific means rather than location-

specific means. We note that California horn snails includes three nominal species (*C. californica*, *C. mazatlantica* and *C. valida*) but we consider these three here as a single, polymorphic species based on identical mitochondrial haplotypes and the same 28S ribosomal RNA genotype (Miura *et al.* 2010).

Table 4.1 – Trematode species of interest

Trematode species name, initials, relative competitive rank (1 = most competitive of 6 species investigated), and number of colonies dissected for 6 species of interest.

Species Name	Initials	Competitive Rank*	n
Parorchis acanthus	PARO	1	13
Himasthla rhigedana	HIMA	2	21
Himasthla sp. B	HIMB	3	29
Acanthoparyphium spinulosum	ACAN	4	37
Cloacitrema michaginensis	CLOA	4	12
Euhaplorchis californiensis	EUHA	5	58
		Total	**170**

Identification and dissection

In the laboratory, the snails were measured with Vernier calipers, processed following Torchin et al (2005), and trematode species were identified following Martin (1972) and Hechinger and Huspeni (unpublished manuscript). We assessed caste allocation, the total number or biomass of soldiers and reproductives within a colony, and soldier deployment, the proportion of soldiers at the front of the snail where trematode invasions occur. For this we utilized snails that had mature, single-species infections of a trematode of interest (Table 1). These snails were extracted from their shell, divided into three sections (mantle, middle, and gonadal region), and soldiers and reproductives were counted in each section (Hechinger *et al.* 2011). While colonies reside in the gonadal region of the snail, initial invasion occurs in the mantle (for species with a short-lived miracidium as the first infective stage) and in the middle (for species with a long-lived egg for the first infective stage). Thus by counting soldiers in each of these sections, we

could calculate two metrics of deployment: the proportion of soldiers in the mantle (PIM) and the proportion of soldiers outside the gonadal region (POG).

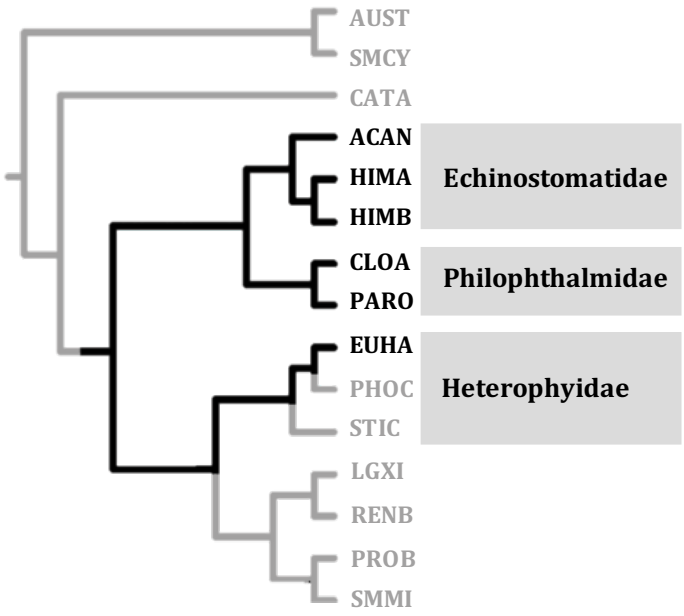

Figure 4.1 – Phylogenetic relationships between trematode species

Phylogeny of trematode species within the California horn snail, modified from Hechinger et al 2009. Species in black denote species of interest for present study. Grey boxes represent a given family.

Estimating soldier parameters

We used individual soldier volume, individual reproductive volume, and soldier mouthpart volume measurements from previous studies (Hechinger et al. 2011, Garcia-Vedrenne et al. 2016, 2017). For HIMB, we had height (h) and width (w) measurements for each of these variables and calculated volume using the equation for the volume of a cylinder ($\pi * \left(\frac{w}{2}\right)^2 h$) as was done previously for the other five trematode species (Garcia-Vedrenne et al. 2016, 2017). We then calculated mean and standard error of individual soldier volume, individual reproductive volume, and soldier mouthpart volume for each species. We also used soldier attack rate estimates from these previous studies (Hechinger et al. 2011, Garcia-Vedrenne et al. 2016, 2017).

In order to estimate the total soldier and reproductive biomass for each colony within a snail that we dissected, we multiplied our soldier and reproductives counts for each colony by mean individual soldier or reproductive volume from previous studies.

Statistical methods

We used one-way ANOVAs to determine if there were significant differences between our species in a number of traits related to caste allocation and distribution. We used the function *qqplot* from the car package in R to determine what distribution fit each variable best. From this, we log-transformed soldier size, pharanx size, total number of soldiers, total soldier biomass, and total reproductive biomass. We ensured model assumptions were met regarding data distributions by examining residual-by-predicted plots and normal quantile plots using *qqnorm* and *qqline* in the car package. For models with significant differences between species, we used the *glht* function from the multcomp package for Tukey HSD post-hoc tests.

Lanchester's laws

We calculated total colony fighting ability for six trematode species using Lanchester's linear and square laws (Lanchester 1916). Lanchester's linear law supposes that colony fighting ability is linearly related to both the number of fighters at the beginning of the fight (m_0), and linearly related to fighting ability (a_m) of individual fighters. In this case, group M should win the fight against group N under these conditions:

$$a_m m_0 > a_n n_0 \quad (1)$$

On the other hand, Lanchester's square law supposes that for battles in an open arena or where group fighting applies, the total number of soldiers should be more important than the individual fighting ability. In this case, the colony fighting ability should be proportional to the square of the size of the fighting group at the beginning of the fight (m_0), but linearly related to the fighting ability (a_m) of individual fighters. In this case, group M should win the fight against group N under these conditions:

$$a_m {m_0}^2 > a_n {n_0}^2 \quad (2)$$

For these analyses, we used total soldiers per colony (for our 170 colonies dissected) for our metric of fighting group size, m_0. For individual fighting ability, a_m, we tried two metrics: mean soldier size and mean soldier size multiplied by mean attack rate. Lanchester's linear and square laws were calculated using these values, giving us four estimates of colony fighting ability for each colony dissected. We then summarized the mean and standard error for these four estimates of colony fighting ability. We compared these estimates of colony fighting ability to the competitive dominance hierarchy.

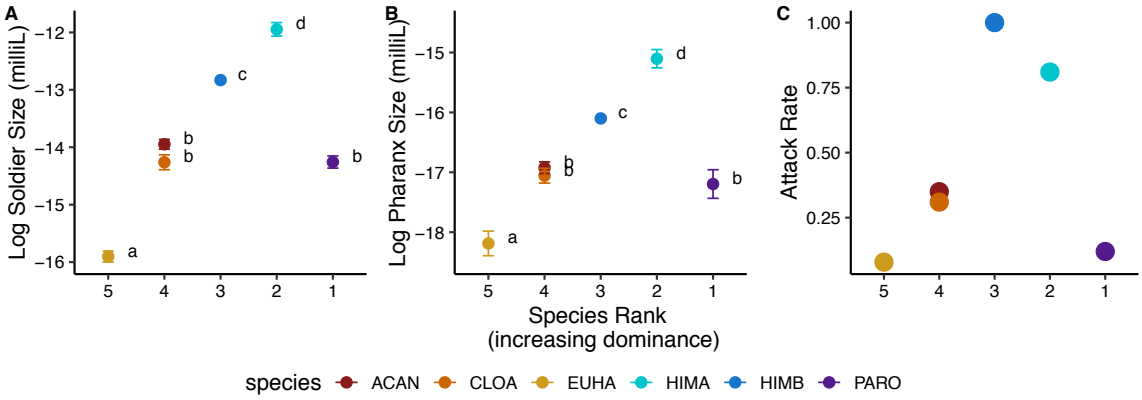

Figure 4.2 – Soldier size, mouthpart (pharanx) size and attack rate observed in previous studies

A) Soldier size, B) mouthpart (pharanx) size and C) attack rate for six trematode species was collated from previous studies (Hechinger et al. 2011, Garcia-Vedrenne et al. 2016, 2017). Error bars represent standard error and letters represent significant differences between species.

RESULTS

First, collating data from previous studies (Hechinger et al. 2011, Garcia-Vedrenne et al. 2016, 2017), we found that species fell into four distinct size classes of soldiers. HIMA soldiers were largest, followed by HIMB, and PARO, ACAN, and CLOA had significantly larger soldiers than EUHA (Figure 4.2A). Pharanx size and soldier size were highly correlated ($r^2 = 0.98$; Figure 4.2B).For attack rate, PARO and

EUHA attacked least often, whereas HIMA and HIMB attacked most often (Figure 4.2C).

We next looked at the allocation of resources between defense and reproduction. We found that the total soldier biomass (the biomass of all soldiers in the colony) was significantly lower for EUHA than all other species (Figure 4.3A). On the other hand, total reproductive biomass was highest for HIMB, then PARO, HIMA, and CLOA, followed by ACAN and finally EUHA (Figure 4.3B). Additionally, in terms of relative allocation of resources (i.e. total biomass) to defense versus reproduction, we found that ACAN had the highest relative allocation of resources to soldier defense, and EUHA had the lowest (Figure 4.3C). PARO, ACAN, and CLOA colonies had the highest total soldiers per colony, HIMB and EUHA had the second highest, and HIMA had the least soldiers (Figure 4.3D).

Finally, we looked at the distribution of soldiers across the snail body (i.e. soldier deployment). We found that HIMA had a significantly higher proportion of soldiers in the mantle (PIM) than the other 5 species (Figure 4.4A). Similarly, HIMA, CLOA, and EUHA had the highest proportion of soldiers outside the gonadal region (POG), followed by PARO, HIMB, and finally ACAN (Figure 4.4B). However, looking at the total number of soldiers in the mantle and outside the gonads revealed that HIMA actually had the fewest soldiers in the mantle or outside the gonadal region, followed by HIMB, ACAN and EUHA, PARO, and finally CLOA (Figure 4.4C-D).

We then tied all of these variables together to determine whether Lanchester's square or linear laws explained the observed dominance hierarchy. When using total soldiers as the fighting group size (m_0) and either soldier size or soldier size * attack rate as the individual fighting ability (a_m), we did not find any trend between estimated group fighting ability (either linear or square) and the competitive rank of our six species (Figure 4.5). We also did not find any relationship between competitive rank and colony fighting ability when using total soldier outside the gonadal region or total soldiers in the mantle.

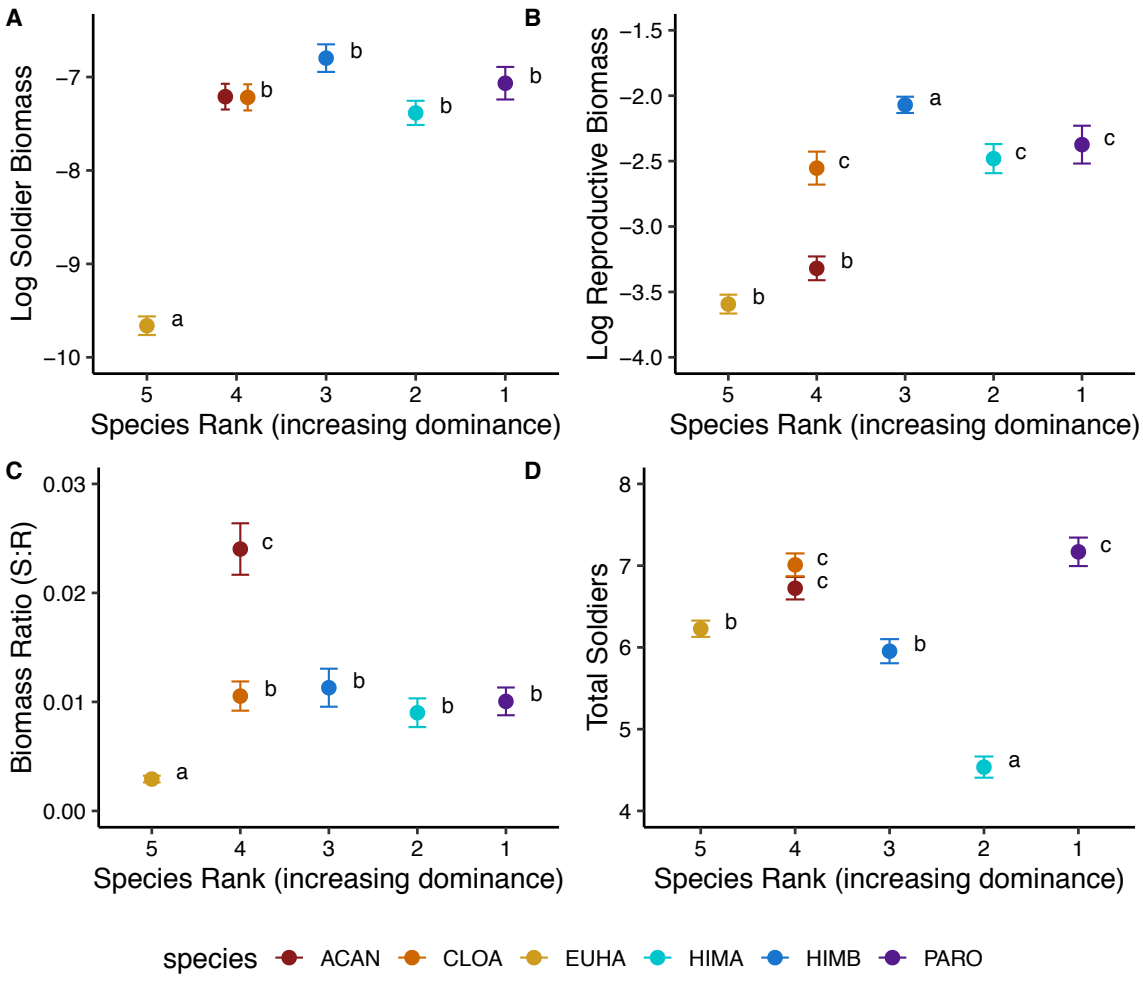

Figure 4.3 – Allocation of resources between defense and reproduction for six species.

A) The total log soldier biomass per colony and B) the total log reproductive biomass per colony. C) The relative allocation of biomass to soldiers versus reproductives. D) The total number of soldiers per colony. Error bars represent standard error and letters represent significant differences between species.

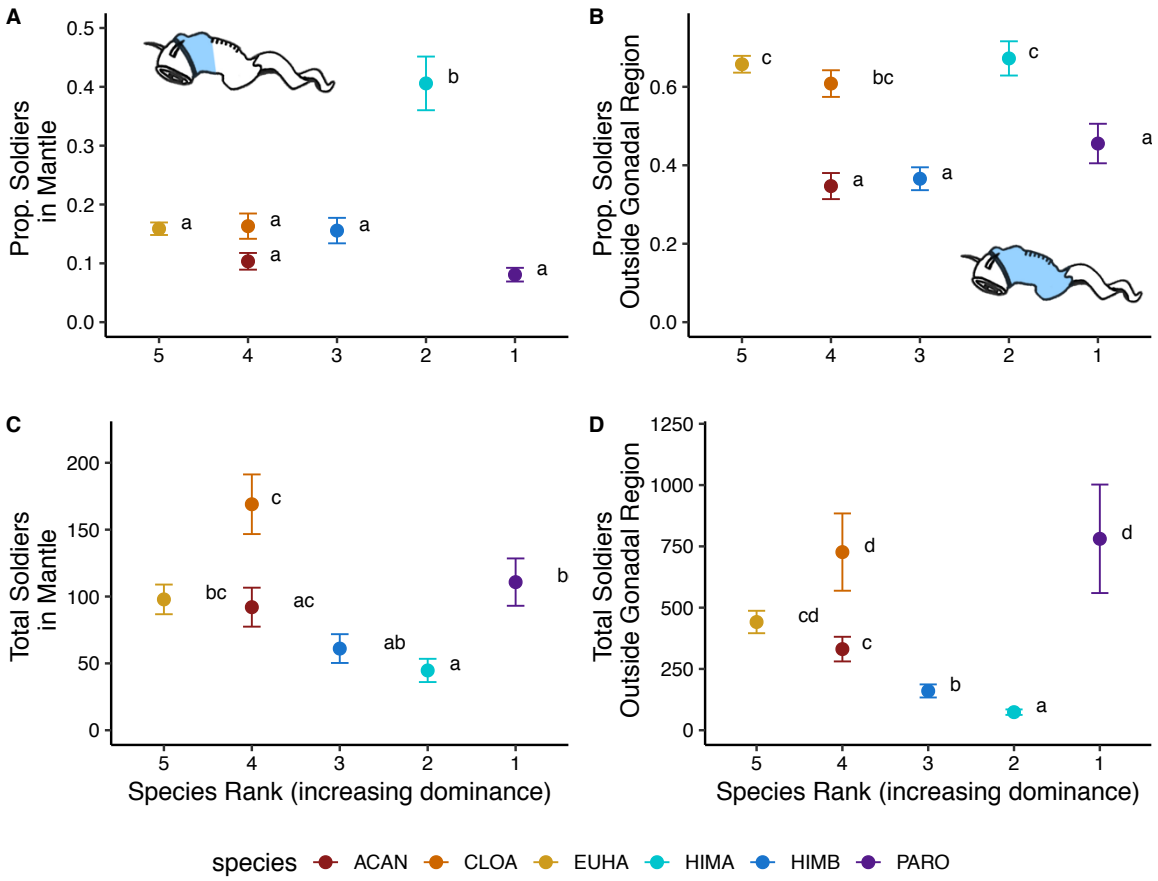

Figure 4.4 – Various metrics of the distribution of soldiers across a colony for six species.

A) The proportion of soldiers in the mantle and B) the proportion of soldiers outside the gonadal region. C) The total number of soldiers in the mantle and D) the total number of soldiers outside the gonadal region. Error bars represent standard error and letters represent significant differences between species.

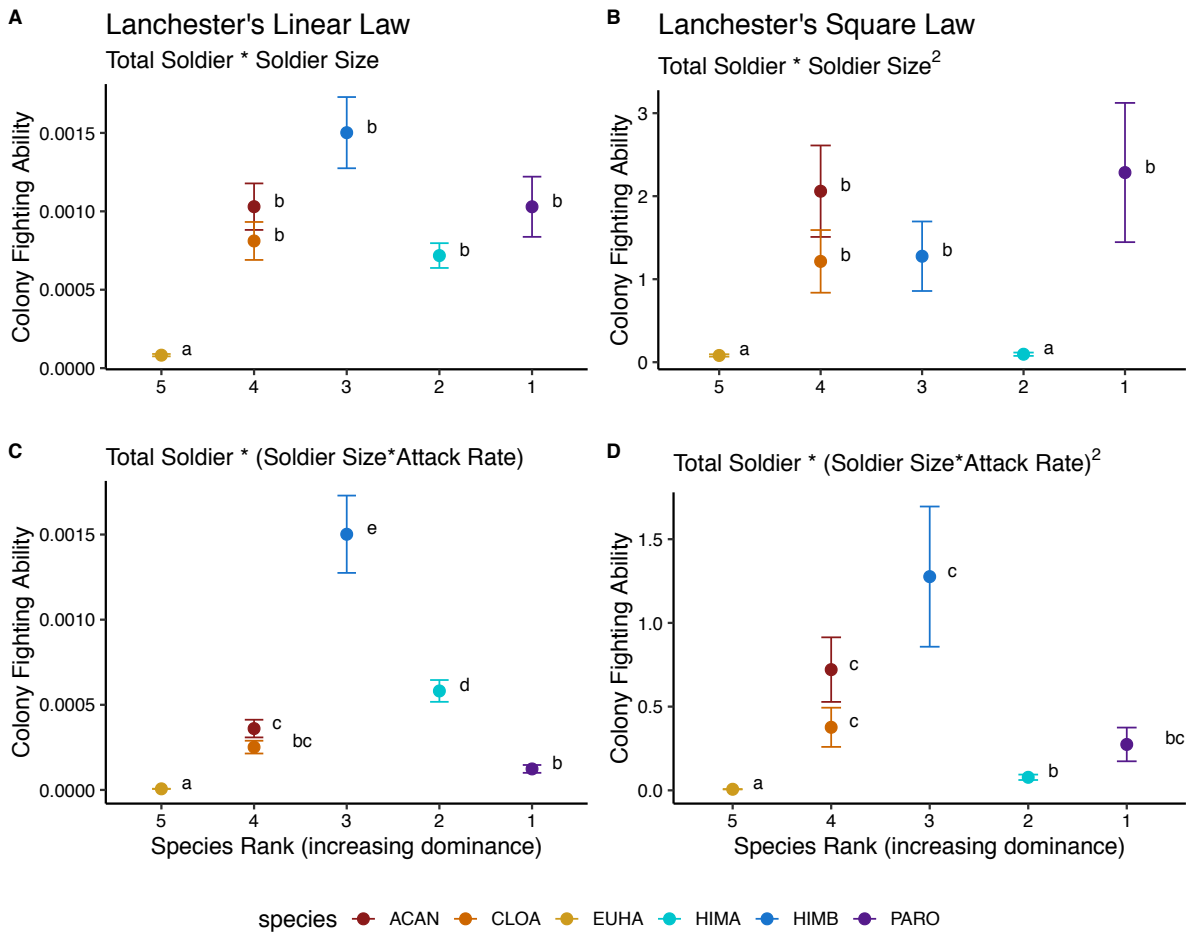

Figure 4.5 – Colony fighting ability across species

Colony fighting ability based on Lanchester's square law (left) and linear law (right) for two different metrics of individual fighting ability: A) soldier size (top), and B) soldier size x attack rate. Error bars represent standard error and letters represent significant differences between species.

DISCUSSION

Here, we combined previous work on individual soldier morphological traits such as body size, mouthpart size and attack rate to estimate the average soldier's individual fighting ability for six species of trematode (Hechinger et al. 2011, Garcia-Vedrenne et al. 2016, 2017). We then utilized our own estimations of total colony size and caste distribution for these six species to estimate colony fighting ability based on Lanchester's laws (Lanchester 1916). Previously, only one species had complete colony censuses (Hechinger *et al.* 2011). Some work had quantified total colony volume for these species, but did not differentiate between castes (Hechinger 2010), while other work estimated the proportion of soldiers and reproductives in each body section, but did not quantify this to allow for complete colony censuses (Garcia-Vedrenne et al. 2016, 2017). Here we show that these six species vary widely in their individual soldier morphological traits and colony attributes.

There was no clear relationship between competitive rank of our six species and either soldier allocation (prediction 1), soldier deployment (prediction 2), or colony fighting ability (prediction 3). This could be for a variety of reasons. Namely, competitive rank is based on a species' ability to defend its colony against invaders and its ability to invade and outcompete an established colony. Here, we exclusively investigate the relative defensibility of a colony. For this study, our data are from mature trematode colonies. However, because invading colonies are composed of a single miracidium (sexually-produced egg that infects snail) and produce very few soldiers to begin with, our understanding of mature colony size, distribution, and soldier phenotypes likely apply to defensibility but not invasibility of a species. Unfortunately, there are few studies on our taxa of interest and none that compare trematode invasion abilities within the *C. californica* host-parasite system. Some studies on different trematode species within our three families of interest (Echinostomatidae, Philophthalmidae, and Heterophyidae) may allow us to glean some relevant information. In general, digenean trematode larvae (all six of our species) are believed to interfere with host internal defense responses, which can in turn affect trematode colonies that are already established within the host

(Interference hypothesis; Lie 1982, Soulsby 1987). Echinostomatidae (e.g. ACAN, HIMA, and HIMB) are thought to particularly affect host hemocytes (Lie *et al.* 1977) and elicit strong immune responses by snail hosts (Loker and Adema 1995). However, larvae of this family appear to rarely be bound by hemocytes, likely due to either a repellent surface or secretory/excretory products (SEP) that repel or damage hemocytes (Loker et al. 1989). This suggests that these strong immune responses can help Echinostomatidae outcompete other (non-Echinostomatidae) established colonies, which are more susceptible to these increased host immune responses. Another study compared Philopthalmidae (e.g. PARO and CLOA) and Heterophyidae (e.g. EUHA) trematode species infecting the common periwinkle snail, *L. littorea,* and found that Philopthalmidae were able to suppress the phagocytic activity of hemocytes more than heterophyids (Iakovleva *et al.* 2006), suggesting that PARO and CLOA may be better able to fight off host immune responses than EUHA. This suggests potentially an important role of differential interference in host immune responses for the competitive dominance hierarchy (Lie et al. 1977, Lie 1982).

Another possible reason that we did not see a relationship between competitive rank and a specific colony metric is that there are trade-offs between different metrics. For instance, soldier size (and by extension mouthpart size) showed a strong increase consistent with increasing competitive rank, for all species except for PARO. PARO and HIMA, which are extremely similar in competitive rank, differ greatly in their soldier and colony attributes, suggesting that they may have very distinct strategies for maintaining dominance. PARO invests in many, minute soldiers that are highly dispersed across the snail and rarely attack heterospecifics. On the other hand, HIMA has a few, massive soldiers that are primarily deployed to the invasion front (outside of the gonadal region) where they attack heterospecifics 100% of the time. Such distinct strategies may be harder to compare directly with the help of Lanchester's laws.

The three Echinostomatidae species (ACAN, HIMB, HIMA) varied significantly for soldier size and total soldiers, suggesting potential niche differentiation between

closely related species. Additionally, ACAN differs substantially from HIMA and HIMB and more closely resembles CLOA, which has the same competitive rank as ACAN.

Previous work suggests that the volume of soldiers can change depending on whether the host is coinfected (or has recently been coinfected) with another trematode species (especially a sporocyst-producing species; Kamiya *et al.* 2013). In this study we do not quantify volume for each colony, but instead utilize volume measurements collected from one estuary as an estimate of volume for each species (Hechinger et al. 2011, Garcia-Vedrenne et al. 2016, 2017). This was due to time constraints and logistic difficulties of working in four separate labs for this work. However, because changes in volume are more likely an outcome of competition (i.e. an increase in food availability due to intraguild predation), rather than an estimation of competitive ability, we do not believe this should affect our across-species comparisons.

This study represents a first step in determining the colony differences that explain the competitive dominance observed in this system. Future work is needed to continue to clarify and validify this competitive hierarchy. For example, more work is needed to differentiate if HIMA and PARO are mutually invasible, or if one species is actually dominant to the other. This would enhance our ability to estimate the parameters that explain this dominance hierarchy. Additionally, it is important that we understand how these species differ in their invasion strategies and abilities. For instance, EUHA is the only species of interest here that infects snails through a log-lived egg, rather than through a shorter-lived, free-swimming miracidium. In what other ways do these species differ in their invasibility? How do these species differ in their secretory/excretory products and effects on host immune function? This would provide us with valuable knowledge, outside this system, on how trematodes invade hosts and how parasite-parasite competition functions.

CONCLUSION

Understanding the factors explaining behavioral dominance is crucial for predicting and mitigating invasive eusocial species such as fire ants, which have tremendous impacts on

native wildlife and local economies (Allen et al. 1994, Pimentel et al. 2005). Additionally, a growing body of literature has focused on social immunity, or how social colonies collectively defend against parasites (Cremer *et al.* 2007). Social trematode colonies represent a more direct analogy for 'social immunity', since trematode soldiers fight off coinfections to protect not only the colony, but also the host (Hechinger *et al.* 2011). In this way, soldiers function similarly to macrophages in the human body (Hechinger *et al.* 2011). An amazing bounty of comparisons between trematode species, with other eusocial species, and with immune systems is likely to yield fruitful insights. All totaled, there are over 20,000 trematode species that infect over 50 families of molluscan first intermediate hosts (Poulin and Morand 2005), in league with the diversity of social insect groups, like ants (Hölldobler and Wilson 1990). The trematode system is rich with opportunity to study the evolution and ecology of sociality, behavioral dominance, and immune defense (Hechinger 2015). Here, we take the first step by demonstrating that soldier investment, deployment, and estimates of colony fighting ability vary significantly between six competing trematode species residing in the California horn snail, but do not explain the dominance hierarchy between these species.

Works Cited

Adler, F. R., E. G. LeBrun, and D. H. Feener. 2007a. Maintaining Diversity in an Ant Community: Modeling, Extending, and Testing the Dominance-Discovery Trade-Off. The American Naturalist 169:323–333.

Adler, P. B., J. HilleRisLambers, and J. M. Levine. 2007b. A niche for neutrality. Ecology Letters 10:95–104.

Aguilera-Olivares, D., V. Palma-Onetto, L. Flores-Prado, V. Zapata, and H. M. Niemeyer. 2017. X-ray computed tomography reveals that intraspecific competition promotes soldier differentiation in a one-piece nesting termite. Entomologia Experimentalis et Applicata 163:26–34.

Allen, C. R., S. Demarais, and R. S. Lutz. 1994. Red imported fire ant impact on wildlife: an overview. The Texas Journal of Science 46:51–59.

Altermatt, F., and M. Holyoak. 2012. Spatial clustering of habitat structure effects patterns of community composition and diversity. Ecology 93:1125–1133.

Ayre, D. J., and R. K. Grosberg. 1996. Effects of social organization on inter-clonal dominance relationships in the sea anemone *Anthopleura elegantissima*. Animal Behaviour 51:1233–1245.

Bengtsson, J. 2009. Applied (meta)community ecology: diversity and ecosystem services at the intersection of local and regional processes. *in* H. A. Vorhoef and P. J. Morin, editors. Metacommunity Ecology. Oxford, UK.

Benton, T. G., M. Solan, J. M. J. Travis, and S. M. Sait. 2007. Microcosm experiments can inform global ecological problems. Trends in Ecology & Evolution 22:516–521.

Binder, B. F. 1988. Intercolonial aggression in the subterranean termite *Heterotermes aureus* (Isoptera: Rhinotermitidae). Psyche 95:123–137.

Borcard, D., and P. Legendre. 2002. All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. Ecological Modelling 153:51–68.

Buck, J. C., R. F. Hechinger, A. C. Wood, T. E. Stewart, A. M. Kuris, and K. D. Lafferty. 2017. Host density increases parasite recruitment but decreases host risk in a snail-trematode system. Ecology 98:2029–2038.

Byers, J. E., Z. C. Holmes, and A. M. H. Blakeslee. 2016. Consistency of trematode infection prevalence in host populations across large spatial and temporal scales. Ecology 97:1643–1649.

Carrara, F., A. Rinaldo, A. Giometto, and F. Altermatt. 2014. Complex interaction of dendritic connectivity and hierarchical patch size on biodiversity in river-like landscapes. The American Naturalist 183:13–25.

Carrara, F., F. Altermatt, I. Rodriguez-Iturbe, and A. Rinaldo. 2012. Dendritic connectivity controls biodiversity patterns in experimental metacommunities. Proceedings of the National Academy of Sciences of the United States of America 109:5761–5766.

Chase, J. M., and M. A. Leibold. 2003. Ecological niches: linking classical and contemporary approaches. University of Chicago Press, Chicago, IL, USA.

Chesson, P. 2000. Mechanisms of Maintenance of Species Diversity. Annual Review of Ecology and Systematics 31:343–366.
Cottenie, K. 2005. Integrating environmental and spatial processes in ecological community dynamics. Ecology Letters 8:1175–1182.
Cremer, S., S. A. O. Armitage, and P. Schmid-Hempel. 2007. Social Immunity. Current Biology 17:R693–R702.
Dray, S., P. Legendre, and P. R. Peres-Neto. 2006. Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). Ecological Modelling 196:483–493.
Duffy, J. E. 1996. Eusociality in a coral-reef shrimp. Nature 381:512–514.
Economo, E. P. 2011. Biodiversity conservation in metacommunity networks: linking pattern and persistence. The American Naturalist 177:E167–80.
Economo, E. P., and T. H. Keitt. 2008. Species diversity in neutral metacommunities: a network approach. Ecology Letters 11:52–62.
Edwards, K. F., and J. J. Stachowicz. 2010. Multivariate trade-offs, succession, and phenological differentiation in a guild of colonial invertebrates. Ecology 91:3146–3152.
Edwards, K. F., and J. J. Stachowicz. 2011. Spatially stochastic settlement and the coexistence of benthic marine animals. Ecology 92:1094–1103.
Fagan, W. F. 2002. Connectivity, fragmentation, and extinction risk in dendritic metapopulations. Ecology 83:3243–3249.
Fellers, J. H. 1987. Interference and exploitation in a guild of woodland ants. Ecology 68:1466–1478.
Fournier, B., N. Mouquet, M. A. Leibold, and D. Gravel. 2016. An integrative framework of coexistence mechanisms in competitive metacommunities. Ecography:1–12.
Francis, L. 1976. Social organization within clones of the sea anemone *Anthopleura elegantissima*. Biological Bulletin 150:361–376.
Fukumori, K., G. Livingston, and M. A. Leibold. 2015. Disturbance mediated colonization-extinction dynamics in experimental protist metacommunities. Ecology 96:3234–3242.
Galaktionov, K. V., and A. A. Dobrovolskij. 2013. The Biology and Evolution of Trematodes.
Garcia-Vedrenne, A. E., A. C. E. Quintana, A. M. DeRogatis, C. M. Dover, M. Lopez, A. M. Kuris, and R. F. Hechinger. 2017. Trematodes with a reproductive division of labour: heterophyids also have a soldier caste and early infections reveal how colonies become structured. International Journal for Parasitology 47:41–50.
Garcia-Vedrenne, A. E., A. C. E. Quintana, A. M. DeRogatis, K. Martyn, A. M. Kuris, and R. F. Hechinger. 2016. Social Organization in Parasitic Flatworms—Four Additional Echinostomoid Trematodes Have a Soldier Caste and One Does Not. Journal of Parasitology 102:11–20.
Gascuel, F., F. Laroche, A.-S. Bonnet-Lebrun, and A. S. L. Rodrigues. 2016. The effects of archipelago spatial structure on island diversity and endemism: predictions from a spatially-structured neutral model. Evolution 70:2657–2666.

Gause, G. F. 1934. Experimental Analysis of Vito Volterra's Mathematical Theory of the Struggle for Existence. Science 79:16–17.
Gravel, D., C. D. Canham, M. Beaudet, and C. Messier. 2006. Reconciling niche and neutrality: the continuum hypothesis. Ecology Letters 9:399–409.
Hanski, I. 1999. Metapopulation Ecology. Oxford University Press, Oxford, UK.
Hanski, I., and M. Gyllenberg. 1997. Uniting Two General Patterns in the Distribution of Species. Science 275:397–400.
Harrison, X. A. 2014. Using observation-level random effects to model overdispersion in count data in ecology and evolution. PeerJ 2:1–19.
Hartig, F. 2019. DHARMa package: Diagnostics for HierArchical Regession Models. R package version 0.2.4.
Hastings, A. 1980. Disturbance, coexistence, history, and competition for space. Theoretical population biology 18:363–373.
Hechinger, R. F. 2010. Mortality affects adaptive allocation to growth and reproduction: field evidence from a guild of body snatchers. BMC evolutionary biology 10:136.
Hechinger, R. F. 2015. Parasites help find universal ecological rules. Proceedings of the National Academy of Sciences of the United States of America 112:1656–1657.
Hechinger, R. F., A. C. Wood, and A. M. Kuris. 2011. Social organization in a flatworm: trematode parasites form soldier and reproductive castes. Proceedings. Biological sciences / The Royal Society 278:656–665.
Hechinger, R. F., A. C. Wood, T. E. Stewart, J. Ashford, A. M. Kuris, and K. D. Lafferty. 2017. Monthly trematode infections of the snail *Cerithideopsis (Cerithidea) californica* at Carpinteria Salt Marsh, California USA, February 2012 to January 2014. U.S. Geological Survey data release.
Hechinger, R. F., and K. D. Lafferty. 2005. Host diversity begets parasite diversity: bird final hosts and trematodes in snail intermediate hosts. Proceedings: Biological Sciences 272:1059–1066.
Hechinger, R. F., K. D. Lafferty, F. T. Mancini, R. R. Warner, and A. M. Kuris. 2009. How large is the hand in the puppet? Ecological and evolutionary factors affecting body mass of 15 trematode parasitic castrators in their snail host. Evolutionary Ecology 23:651–667.
Holway, D. A., and T. J. Case. 2001. Effects of colony-level variation on competitive ability in the invasive Argentine ant. Animal Behaviour 61:1181–1192.
Holway, D., A. Suarez, and T. Case. 1998. Loss of intraspecific aggression in the success of a widespread invasive social insect. Science 282:949–952.
Holyoak, M., M. A. Leibold, and R. D. Holt. 2005. Metacommunities: spatial dynamics and ecological communities. University of Chicago Press, Chicago, IL, USA.
Hölldobler, B., and E. O. Wilson. 1990. The Ants. Harvard University Press.
Hubbell, S. P. 2001. The unified neutral theory of biodiversity and biogeography. Princeton University Press, Princeton, NJ, USA.
Huspeni, T. C. 2000. A molecular genetic analysis of host specificity, continental geography, and recruitment dynamics of a larval trematode in a salt marsh snail. University of California, Santa Barbara.

Hutchinson, G. E. 1957. Cold spring harbor symposium on quantitative biology. Concluding remarks.
Hutchinson, G. E. 1959. Homage to Santa Rosalia or why are there so many kinds of animals? American Naturalist 93:145–159.
Iakovleva, N. V., T. G. Shaposhnikova, and A. M. Gorbushin. 2006. Rediae of echinostomatid and heterophyid trematodes suppress phagocytosis of haemocytes in *Littorina littorea* (Gastropoda: Prosobranchia). Experimental Parasitology 113:24–29.
Jarvis, J. U. 1981. Eusociality in a mammal: cooperative breeding in naked mole-rat colonies. Science 212:571–573.
Jahn, T. L., Jahn, F. F. & Bovee, E. C. *How to Know the Protozoa*. (McGraw-Hill, 1978).
Kamiya, T., K. O'Dwyer, J. Nuy, and R. Poulin. 2013. What determines the growth of individual castes in social trematodes? Evolutionary Ecology 27:1235–1247.
Kaspari, M., and M. M. Byrne. 1995. Caste allocation in litter *Pheidole*: lessons from plant defense theory. Behavioral Ecology and Sociobiology 37:255–263.
Keeney, D. B., T. M. King, D. L. Rowe, and R. Poulin. 2009. Contrasting mtDNA diversity and population structure in a direct-developing marine gastropod and its trematode parasites. Molecular Ecology 18:4591–4603.
Kilham, S. S., D. A. Kreeger, S. G. Lynn, C. E. Goulden, and L. Herrera. 1998. COMBO: a defined freshwater culture medium for algae and zooplankton. Hydrobiologia 377:147–159.
Kneitel, J. M., and J. M. Chase. 2004. Trade-offs in community ecology: linking spatial scales and species coexistence. Ecology Letters 7:69–80.
Kuris, A. 1990. Guild structure of larval trematodes in molluscan hosts: prevalence, dominance and significance of competition. Pages 69–100 *in* Parasite Communities: Patterns and Processes. Springer, Dordrecht, Dordrecht.
Kuris, A. M., and K. D. Lafferty. 1994. Community structure: larval trematodes in snail hosts. Annual Review of Ecology and Systematics 25:189–217.
Lafferty, K. D. 2001. Birds at a Southern California beach: seasonality, habitat use and disturbance by human activity. Biodiversity & Conservation 10:1949–1962.
Lafferty, K. D., D. T. Sammond, and A. M. Kuris. 1994. Analysis of larval trematode communities. Ecology 75:2275–2285.
Lagrue, C., C. D. MacLeod, L. Keller, and R. Poulin. 2018. Caste ratio adjustments in response to perceived and realised competition in parasites with division of labour. The Journal of animal ecology 87:1429–1439.
Lanchester, F. W. 1916. Aircraft in Warfare. Constable, London.
Legendre, P., and E. Gallagher. 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129:271–280.
Leibold, M. A. 2011. The metacommunity concept and its theoretical underpinnings. *in* S. M. Scheiner and M. R. Willig, editors. The Theory of Ecology. University of Chicago Press, Chicago, IL, USA.
Leibold, M. A., and J. M. Chase. 2017. Metacommunity Ecology. Princeton University Press, Princeton, NJ, USA.

Leibold, M. A., and M. A. McPeek. 2006. Coexistence of the niche and neutral perspectives in community ecology. Ecology 87:1399–1410.
Leibold, M. A., and N. Loeuille. 2015. Species sorting and patch dynamics in harlequin metacommunities affect the relative importance of environment and space. Ecology 96:3227–3233.
Leibold, M. A., J. M. Chase, and S. K. M. Ernest. 2017. Community assembly and the functioning of ecosystems: how metacommunity processes alter ecosystems attributes. Ecology 98:909–919.
Leibold, M. A., M. Holyoak, N. Mouquet, P. Amarasekare, J. M. Chase, M. F. Hoopes, R. D. Holt, J. B. Shurin, R. Law, D. Tilman, M. Loreau, and A. Gonzalez. 2004. The metacommunity concept: a framework for multi-scale community ecology. Ecology Letters 7:601–613.
Leroux, S. J., C. H. Albert, A.-S. Lafuite, B. Rayfield, S. Wang, and D. Gravel. 2016. Structural uncertainty in models projecting the consequences of habitat loss and fragmentation on biodiversity. Ecography:1–12.
Levin, S. A. 1970. Community equilibria and stability, and an extension of the competitive exclusion principle. Biological Bulletin 104:413–423.
Levins, R. 1969. Some Demographic and Genetic Consequences of Environmental Heterogeneity for Biological Control. Bulletin of the Entomological society of America 15:237–240.
Lie, K. J. 1982. Survival of *Schistosoma mansoni* and other trematode larvae in the snail *Biomphalaria glabrata.* A discussion of the interference theory. Tropical and geographical medicine 34:111–122.
Lie, K. J., D. Heyneman, and C. S. Richards. 1977. Studies on resistance in snails: Interference by nonirradiated echinostome larvae with natural resistance to *Schistosoma mansoni* in *Biomphalaria glabrata*. Journal of Invertebrate Pathology 29:118–125.
Lloyd, M. M., and R. Poulin. 2013a. Reproduction and caste ratios under stress in trematode colonies with a division of labour. Parasitology 140:825–832.
Lloyd, M. M., and R. Poulin. 2013b. Multi-clone infections and the impact of intraspecific competition on trematode colonies with a division of labour. Parasitology 141:304–310.
Lloyd, M. M., and R. Poulin. 2014. Geographic variation in caste ratio of trematode colonies with a division of labour reflect local adaptation. Parasitology Research 113:2593–2602.
Logue, J. B., N. Mouquet, H. Peter, H. Hillebrand, The Metacommunity Working Group. 2011. Empirical approaches to metacommunities: a review and comparison with theory. Trends in Ecology & Evolution 26:482–491.
Loker, E. S., and C. M. Adema. 1995. Schistosomes, echinostomes and snails: comparative immunobiology. Animal Behaviour 11:120–124.
Loker, E. S., M. E. Boston, and C. J. Bayne. 1989. Differential adherence of M line *Biomphalaria glabrata* hemocytes to *Schistosoma mansoni* and *Echinostoma*

paraensei larvae, and experimental manipulation of hemocyte binding. Journal of Invertebrate Pathology 54:260–268.
Lynn, D. *The Ciliated Protozoa*. (Springer Netherlands, 1979).
MacArthur, R. H. 1969. Patterns of communities in the tropics. Biological Journal of the Linnean Society 1:19–30.
MacLeod, C., R. Poulin, and C. Lagrue. 2018. Save your host, save yourself? Caste-ratio adjustment in a parasite with division of labor and snail host survival following shell damage. Ecology and Evolution 8:1615–1625.
Margules, C. R., and R. L. Pressey. 2000. Systematic conservation planning. Nature 405:243–253.
McGlynn, T. P. 2000. Do Lanchester's laws of combat describe competition in ants? Biological Journal of the Linnean Society 11:686–690.
Miura, O., M. E. Torchin, A. M. Kuris, R. F. Hechinger, and S. Chiba. 2006. Introduced cryptic species of parasites exhibit different invasion pathways. Proceedings of the National Academy of Sciences 103:19818–19823.
Miura, O., M. E. Torchin, and E. Bermingham. 2010. Molecular phylogenetics reveals differential divergence of coastal snails separated by the Isthmus of Panama. Molecular Phylogenetics and Evolution 56:40–48.
Moilanen, A., A. M. A. Franco, R. I. Early, R. Fox, B. Wintle, and C. D. Thomas. 2005. Prioritizing multiple-use landscapes for conservation: methods for large multi-species planning problems. Proceedings: Biological Sciences 272:1885–1891.
Mordecai, E. A., A. G. Jaramillo, J. E. Ashford, R. F. Hechinger, and K. D. Lafferty. 2016. The role of competition--colonization tradeoffs and spatial heterogeneity in promoting trematode coexistence. Ecology 97:1484–1496.
Mouquet, N., and M. Loreau. 2003. Community patterns in source-sink metacommunities. dx.doi.org 162:544–557.
Mouquet, N., D. Gravel, F. Massol, and V. Calcagno. 2012. Extending the concept of keystone species to communities and ecosystems. Ecology Letters 16:1–8.
Mouritsen, K. N., and C. Andersen. 2017. Worms at war: interspecific parasite competition and host resources alter trematode colony structure and fitness. Parasitology 144:1530–1542.
Nee, S., and R. M. May. 1992. Dynamics of metapopulations: habitat destruction and competitive coexistence. Annales Zoologici Fennici 61:37.
O'Riain, M. J., J. U. M. Jarvis, R. Alexander, R. Buffenstein, and C. Peeters. 2000. Morphological castes in a vertebrate. Proceedings of the National Academy of Sciences 97:13194–13197.
Oster, G. F., and E. O. Wilson. 1978. Caste and ecology in the social insects. Monographs in population biology 12:1–352.
Paine, R. T. 1966. Food web complexity and species diversity. American Naturalist.
Paine, R. T. 1969. The Pisaster-Tegula interaction: prey patches, predator food preference, and intertidal community structure. Ecology 50:950.

Paine, R. T. 2010. Macroecology: Does It Ignore or Can It Encourage Further Ecological Syntheses Based on Spatially Local Experimental Manipulations? dx.doi.org 176:385–393.
Palmer, T. M. 2004. Wars of attrition: colony size determines competitive outcomes in a guild of African acacia ants. Animal Behaviour 68:993–1004.
Palmer, T. M., T. P. Young, M. L. Stanton, and E. Wenk. 2000. Short-term dynamics of an acacia ant community in Laikipia, Kenya. Oecologia 123:425–435.
Passera, L., E. Roncin, B. Kaufmann, and L. Keller. 1996. Increased soldier production in ant colonies exposed to intraspecific competition. Nature 379:630–631.
Peres-Neto, P. R., P. Legendre, S. Dray, and D. Borcard. 2006. Variation partitioning of species data matrices: estimation and comparison of fractions. Ecology 87:2614–2625.
Pike, N. 2007. Specialised placement of morphs within the gall of the social aphid *Pemphigus spyrothecae.* BMC evolutionary biology 7:18.
Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52:273–288.
Poulin, R., and S. Morand. 2005. Parasite Biodiversity. Smithsonian Institution, Washington, D.C.
Powell, S., M. Donaldson-Matasci, A. Woodrow-Tomizuka, and A. Dornhaus. 2017. Context-dependent defences in turtle ants: Resource defensibility and threat level induce dynamic shifts in soldier deployment. Functional Ecology 31:2287–2298.
Resetarits, W. J., Jr, and A. Silberbush. 2015. Local contagion and regional compression: habitat selection drives spatially explicit, multiscale dynamics of colonisation in experimental metacommunities. Ecology Letters 19:191–200.
Schiesari, L., K. L. Prado, M. A. Leibold, C. H. Albert, P. H. S. Brancalion, M. Cabeza, and R. Coutinho. 2002. The why, when, and how of applied metaecology.
Schoener, T. W. 1983. Field experiments on interspecific competition. Biological Bulletin 122:240–285.
Shibao, H. 1998. Social structure and the defensive role of soldiers in a eusocial bamboo aphid, *Pseudoregma bambucicola* (Homoptera: Aphididae): A test of the defence-optimization hypothesis. Population Ecology 40:325–333.
Shibao, H. 1999. Reproductive schedule and factors affecting soldier production in the eusocial bamboo aphid *Pseudoregma bambucicola* (Homoptera, Aphididae). Insectes Sociaux 46:378–386.
Soulsby, E. 1987. Immune responses in parasitic infections: immunology, immunopathology, and immunoprophylaxis.
Sousa, W. P. 1993. Interspecific antagonism and species coexistence in a diverse guild of larval trematode parasites. Ecology.
Stanton, M. L., T. M. Palmer, and T. P. Young. 2002. Competition–colonization trade-offs in a guild of African acacia-ants. Ecological Monographs 72:347–363.
Tilman, D. 1982. Resource competition and community structure. Monographs in population biology 17:1–296.

Tilman, D. 1994. Competition and Biodiversity in Spatially Structured Habitats. Ecology 75:2–16.
Torchin, M. E., O. Miura, and R. F. Hechinger. 2015. Parasite species richness and intensity of interspecific interactions increase with latitude in two wide-ranging hosts. Ecology 96:3033–3042.
Traniello, J. F. A. 1981. Enemy deterrence in the recruitment strategy of a termite: Soldier-organized foraging in *Nasutitermes costalis*. Proceedings of the National Academy of Sciences 78:1976–1979.
Troll, C. 1939. Luftbildplan und ökologische Bodenforschung. Zeitschrift der Gesellschaft für Erdkunde:241–298.
Urban, D., and T. Keitt. 2001. Landscape Connectivity: a Graph-Theoretic Perspective. Ecology 82:1205–1218.
Vepsäläinen, K., R. Savolainen, J. Tiainen, and J. Vilén. 2000. Successional changes of ant assemblages: from virgin and ditched bogs to forests. Annales Zoologici Fennici 37:135–149.
Walker, J., and J. Stamps. 1986. A test of optimal caste ratio theory using the ant *Camponotus (Colobopsis) impressus*. Ecology 67:1052–1062.
Wills, B. D., C. S. Moreau, and B. D. Wray. 2014. Body size variation and caste ratios in geographically distinct populations of the invasive big-headed ant, *Pheidole megacephala* (Hymenoptera: Formicidae). Biological Journal of the Linnean Society 113:423–438.
Wills, B. D., S. Powell, M. D. Rivera, and A. V. Suarez. 2018. Correlates and Consequences of Worker Polymorphism in Ants. Annual Review of Entomology 63:575–598.
Wilson, E. O., and R. H. MacArthur. 1967. The theory of island biogeography. Princeton University Press, Princeton, NJ, USA.
Wilson, E. O., and R. Southwood. 1990. Success and dominance in ecosystems: the case of the social insects. Ecology Institute, Oldendorf/Luhe, Germany.
Yang, A. S., C. H. Martin, and H. F. Nijhout. 2004. Geographic Variation of Caste Structure among Ant Populations. Current Biology 14:514–519.